

NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

RELATED APPLICATIONS

This application claims priority from Applications U.S.S.N. 09/823,172 filed March 29, 2001, U.S.S.N. 60/193,664, filed March 31, 2000; U.S.S.N. 60/194,614, filed April 5, 2000; U.S.S.N. 60/195,063, filed April 6, 2000; U.S.S.N. 60/195,066, filed April 6, 2000; U.S.S.N. 60/195,067, filed April 6, 2000; U.S.S.N. 60/195,068, filed April 6, 2000; U.S.S.N. 60/195,069, filed April 6, 2000; U.S.S.N. 60/195,070, filed April 6, 2000; U.S.S.N. 60/195,510, filed April 6, 2000; U.S.S.N. 60/219,855, filed on July 21, 2001; U.S.S.N. 60/221,284, filed on July 27, 2000; U.S.S.N. 60/221,325 filed July 28, 2000; U.S.S.N. 60/224,588 filed August 11, 2000; U.S.S.N. 60/239,613, filed October 11, 2000; U.S.S.N. ??????, filed January 18, 2001; Express Mail Number: EL58233735US, filed January 23, 2001; Express Mail Number: EL585233758US, filed January 23, 2001; and Express Mail Number: EL585234016US, filed January 30, 2001; each of which is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides. More particularly, the invention relates to nucleic acids encoding novel G-protein coupled receptor (GPCR) polypeptides, as well as vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and polypeptides.

SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of nucleic acid sequences encoding novel polypeptides. The novel nucleic acids and polypeptides are referred to herein as GPCR_X, or GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, GPCR7, GPCR8, and GPCR9 nucleic acids and polypeptides. These nucleic acids and polypeptides, as well as derivatives, homologs, analogs and fragments thereof, will hereinafter be collectively designated as "GPCR_X" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated GPCR_X nucleic acid molecule encoding a GPCR_X polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids disclosed in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84. In some embodiments, the GPCR_X nucleic acid molecule will hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of a GPCR_X nucleic acid sequence. The invention also includes an isolated nucleic acid that encodes a GPCR_X polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide comprising the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a GPCR_X nucleic acid (*e.g.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84) or a complement of said oligonucleotide.

Also included in the invention are substantially purified GPCR_X polypeptides (SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85). In certain embodiments, the GPCR_X polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human GPCR_X polypeptide.

The invention also features antibodies that immunoselectively bind to GPCR_X polypeptides, or fragments, homologs, analogs or derivatives thereof.

In another aspect, the invention includes pharmaceutical compositions that include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a GPCR_X nucleic acid, a GPCR_X polypeptide, or an antibody specific for a GPCR_X polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a GPCR_X nucleic acid, under conditions allowing for expression of the GPCR_X polypeptide encoded by the DNA. If desired, the GPCR_X polypeptide can then be recovered.

In another aspect, the invention includes a method of detecting the presence of a GPCR_X polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby identifying the GPCR_X polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of a GPCR_X.

Also included in the invention is a method of detecting the presence of a GPCR_X nucleic acid molecule in a sample by contacting the sample with a GPCR_X nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to a GPCR_X nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of a GPCR_X polypeptide by contacting a cell sample that includes the GPCR_X polypeptide with a compound that binds to the GPCR_X polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, or other disorders related to cell signal processing and metabolic pathway modulation. The therapeutic can be, *e.g.*, a GPCR_X nucleic acid, a GPCR_X polypeptide, or a GPCR_X-specific antibody, or biologically-active derivatives or fragments thereof.

For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: developmental diseases, MHCII and III diseases (immune diseases), taste and scent detectability Disorders, Burkitt's lymphoma, corticoneurogenic disease, signal transduction pathway disorders, Retinal diseases including those involving photoreception, Cell growth rate disorders; cell shape disorders, feeding disorders; control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2),

pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidoluysian atrophy (DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding GPCR_X may be useful in gene therapy, and GPCR_X may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders or other disorders related to cell signal processing and metabolic pathway modulation. The method includes contacting a test compound with a GPCR_X polypeptide and determining if the test compound binds to said GPCR_X polypeptide. Binding of the test compound to the GPCR_X polypeptide indicates the

test compound is a modulator of activity, or of latency or predisposition to the
aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of
activity, or of latency or predisposition to an disorders or syndromes including, *e.g.*, diabetes,
5 metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting
disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious
disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders,
Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders
or other disorders related to cell signal processing and metabolic pathway modulation by
10 administering a test compound to a test animal at increased risk for the aforementioned
disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a
GPCRX nucleic acid. Expression or activity of GPCR_X polypeptide is then measured in the
test animal, as is expression or activity of the protein in a control animal which
recombinantly-expresses GPCR_X polypeptide and is not at increased risk for the disorder or
15 syndrome. Next, the expression of GPCR_X polypeptide in both the test animal and the
control animal is compared. A change in the activity of GPCR_X polypeptide in the test
animal relative to the control animal indicates the test compound is a modulator of latency of
the disorder or syndrome.

In yet another aspect, the invention includes a method for determining the presence of
20 or predisposition to a disease associated with altered levels of a GPCR_X polypeptide, a
GPCRX nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes
measuring the amount of the GPCR_X polypeptide in a test sample from the subject and
comparing the amount of the polypeptide in the test sample to the amount of the GPCR_X
polypeptide present in a control sample. An alteration in the level of the GPCR_X polypeptide
25 in the test sample as compared to the control sample indicates the presence of or
predisposition to a disease in the subject. Preferably, the predisposition includes, *e.g.*,
diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X,
anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes,
obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative
30 disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic
disorders. Also, the expression levels of the new polypeptides of the invention can be used in
a method to screen for various cancers as well as to determine the stage of cancers.

In a further aspect, the invention includes a method of treating or preventing a
pathological condition associated with a disorder in a mammal by administering to the

subject a GPCR_X polypeptide, a GPCR_X nucleic acid, or a GPCR_X-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

In yet another aspect, the invention can be used in a method to identify the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode novel polypeptides. The novel nucleic acids and their encoded polypeptides are referred to individually as GPCR₁, GPCR₂, GPCR₃, GPCR₄, GPCR₅, GPCR₆, GPCR₇, GPCR₈, GPCR₉, and GPCR₁₀. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "GPCR_X".

The novel GPCR_X nucleic acids of the invention include the nucleic acids whose sequences are provided in Tables 1A, 1E, 1I, 2A, 2I, 3A, 4A, 4C, 4G, 5A, 5C, 5G, 6A, 7A, 8A, 9A, 10A, 10C and 10F, inclusive ("Tables 1A - 10F"), or a fragment, derivative, analog or homolog thereof. The novel GPCR_X proteins of the invention include the protein fragments whose sequences are provided in Tables 1B, 1F, 1J, 2B, 2J, 3B, 4B, 4H, 5B, 5D,

5H, 6B, 7B, 8B, 9B, 10B, 10D, and 10G, inclusive ("Tables 1B - 10G"). The individual GPCR nucleic acids and proteins are described below. Within the scope of this invention is a method of using these nucleic acids and peptides in the treatment or prevention of a disorder related to cell signaling or metabolic pathway modulation.

5 G-Protein Coupled Receptor proteins (GPCRs) have been identified as a large family of G protein-coupled receptors in a number of species. These receptors share a seven transmembrane domain structure with many neurotransmitter and hormone receptors, and are likely to underlie the recognition and G-protein-mediated transduction of various signals. Human GPCR generally do not contain introns and belong to four different gene subfamilies, displaying great sequence variability. These genes are dominantly expressed in olfactory epithelium. See, *e.g.*, Ben-Arie et al., Hum. Mol. Genet. 1994 3:229-235; and, Online Mendelian Inheritance in Man (OMIM) entry # 164342 (<http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?>).

15 The olfactory receptor (OR) gene family constitutes one of the largest GPCR multigene families and is distributed among many chromosomal sites in the human genome. See Rouquier et al., Hum. Mol. Genet. 7(9):1337-45 (1998); Malnic et al., Cell 96:713-23 (1999). Olfactory receptors constitute the largest family among G protein-coupled receptors, with up to 1000 members expected. See Vanderhaeghen et al., Genomics 39(3):239-46 (1997); Xie et al., Mamm. Genome 11(12):1070-78 (2000); Issel-Tarver et al., Proc. Natl. Acad. Sci. USA 93(20):10897-902 (1996). The recognition of odorants by olfactory receptors is the first stage in odor discrimination. See Krautwurst et al., Cell 95(7):917-26 (1998); Buck et al., Cell 65(1):175-87 (1991). Many ORs share some characteristic sequence motifs and have a central variable region corresponding to a putative ligand binding site. See Issel-Tarver et al., Proc. Natl. Acad. Sci. USA 93:10897-902 (1996).

25 Other examples of seven membrane spanning proteins that are related to GPCRs are chemoreceptors. See Thomas et al., Gene 178(1-2):1-5 (1996). Chemoreceptors have been identified in taste, olfactory, and male reproductive tissues. See *id.*; Walensky et al., J. Biol. Chem. 273(16):9378-87 (1998); Parmentier et al., Nature 355(6359):453-55 (1992); Asai et al., Biochem. Biophys. Res. Commun. 221(2):240-47 (1996).

30 GPCR1

GPCR1 includes a family of three novel G-protein coupled receptor ("GPCR") proteins disclosed below. The disclosed proteins have been named GPCR1a, GPCR1b, and GPCR1c and are related to olfactory receptors.

GPCR1a

A disclosed GPCR1a nucleic acid of 1050 nucleotides is shown in Table 1A. The disclosed GPCR1a open reading frame ("ORF") begins at the ATG initiation codon at nucleotides 6-8, shown in bold in Table 1A. The encoded polypeptide is alternatively referred to herein as GPCR1a or as rp11-507n20_A. The disclosed GPCR1a ORF terminates at a TGA codon at nucleotides 1044-1046. As shown in Table 1A, putative untranslated regions 5' to the start codon and 3' to the stop codon are underlined, and the start and stop codons are in bold letters.

Table 1A. GPCR1a nucleotide sequence (SEQ ID NO:1).

CCGCCATGTACAACGGGTCGTGCTGCCGCATCGAGGGGACACCATCTCCAGGTGATGCCGCCGTGCTCATTGTG
GCCTTTGTGCTGGGCGCACTAGGCAATGGGGTCGCCCTGTGTGGTTTCTGCTCCACATGAAGACCTGGAAGCCCAG
CACTGTTTACCTTTTCAATTTGGCCGTGGCTGATTTCTCTCTATGATCTGCCTGCCCTTTTCGGACAGACTATTACC
TCAGACGTAGACACTGGGCTTTTGGGGACATTCCCTGCCGAGTGGGGCTCTTCACGTTGGCCATGAACAGGGCCGGG
AGCATCGTGTTCCTTACGGTGGTGGCTGCGGACAGGTATTTCAAAGTGGTCCACCCCAACACGCGGTGAACACTAT
CTCCACCCGGGTGGCGGTGGCATCGTCTGCACCTGTGGGCCCTGGTCATCCTGGGAACAGTGTATCTTTTGCTGG
AGAACCATCTCTGCGTGCAAGAGACGGCCGTCTCTGTGAGAGCTTCATCATGGAGTCGGCCAATGGCTGGCATGAC
ATCATGTTCCAGCTGGAGTTCCTTATGCCCCCTCGGCATCATCTTATTTTGCTCCTTCAAGATTGTTTGGAGCCTGAG
GCGGAGGCAGCAGCTGGCCAGACAGGCTCGGATGAAGAAGGCGACCCGGTTCATCATGGTGGTGGCAATTGTGTTCA
TCACATGCTACCTGCCAGCGTGTCTGTAGACTCTATTCTCTGGACGGTGCCTCGAGTGCTGCGATCCCTCT
GTCCATGGGGCCCTGCACATAACCCTCAGCTTCACCTACATGAACAGCATGCTGGATCCCCTGGTGTATTATTTTTC
AAGCCCTCCTTTCCCAAATTTACAACAAGCTCAAATCTGCAGTCTGAAACCAAGCAGCCAGGACACTCAAAAA
CACAAAGGCCGGAAGAGATGCCAATTTGAACTCGGTGCGAGGAGTTGCATCAGTGTGGCAATAGTTTCCAAAGC
CAGTCTGATGGGCAATGGGATCCCAACATTGTTGAGTGGCACTGAACAA

A disclosed encoded GPCR1a protein has 346 amino acid residues, referred to as the GPCR1a protein. The GPCR1a protein was analyzed for signal peptide prediction and cellular localization. SignalP results predict that GPCR1a is cleaved between position 32 and 33 of SEQ ID NO:2, *i.e.*, in the amino acid sequence ALG-NG. Psort and Hydropathy profiles also predict that GPCR1 contains a signal peptide and is likely to be localized in the plasma membrane (Certainty 0.6400). The disclosed GPCR1 polypeptide sequence is presented in Table 1B using the one-letter amino acid code.

Table 1B. Encoded GPCR1a protein sequence (SEQ ID NO:2).

MYNGSCCRIEGDTISQVMPPLLIIVAFVLGALGNGVALCGFCFHMKTWKPSTVYLFNLAVADFLLMICLPFRTDYYLR
RRHWAFGDIPCRVGLFTLAMNRAGSIVFLTVVAADRYFKVPHAVNTISTRVAAGIVCTLWALVILGTVYLLLEN
HLCVQETAVSCSFIMESANGWHDIMFQLEFFMPLGIILFCFSFKIVWSLRRRQQLARQARMKKATRFIMVVAIVFIT
CYLPSVSARLYFLWTVSSACDPSVHGALHITLSFTYMNMSMLDPLVYVFSSPSFPKFKYNLKLKICSLKPKQPGHSKTQ
RPEEMPISNLGRRSCISVANSFQSQSDGQWDPHIVEWH

GPCR1a was initially identified on chromosome 12 with a TblastN analysis of a proprietary sequence file for a G-protein coupled receptor probe or homolog, which was run

against the Genomic Daily Files made available by GenBank. A proprietary software program (GenScan™) was used to further predict the nucleic acid sequence and the selection of exons. The resulting sequences were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein.

A region of the GPCR1a nucleic acid sequence has 517 of 746 bases (69%) identical to a *Homo sapiens* GPCR mRNA (GENBANK-ID: HUMHM74|acc:D10923), with an E-value of 1.9×10^{-71} . In all BLAST alignments herein, the “E-value” or “Expect” value is a numeric indication of the probability that the aligned sequences could have achieved their similarity to the BLAST query sequence by chance alone, within the database that was searched. For example, the probability that the subject (“Sbjct”) retrieved from the GPCR1a BLAST analysis, *e.g.*, the *Homo sapiens* GPCR, matched the Query GPCR1a sequence purely by chance is 1.9×10^{-71} .

A BLASTX search was performed against public protein databases. The full amino acid sequence of the protein of the invention was found to have 178 of 339 amino acid residues (52%) identical to, and 227 of 339 residues (66%) positive with, the 387 amino acid residue protein from *species* (ptnr: SWISSPROT-ACC:P49019).

The amino acid sequence of GPCR1a also had high homology to other proteins as shown in table 1C.

Table 1C. BLASTX alignments of GPCR1a

Sequences producing High-scoring Segment Pairs:			Smallest
	Reading Frame	High Score	Sum Prob. P (N)
ptnr:SWISSPROT-ACC:O00270 PROBABLE G PROTEIN-COUPLED R...	+3	447	2.6e-41
ptnr:TREMBLNEW-ACC:AAF26668 G PROTEIN COUPLED RECEPTOR...	+3	441	1.1e-40
ptnr:SWISSPROT-ACC:P34996 P2Y PURINOCEPTOR 1 (ATP RECE...	+3	357	8.8e-32
ptnr:SWISSPROT-ACC:P49652 P2Y PURINOCEPTOR 1 (ATP RECE...	+3	357	8.8e-32

GPCR1b

A disclosed GPCR1b (also referred to as rp11-507n20_A_da1) nucleic acid of 1050 nucleotides is shown in Table 1D. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 6-8 and ending with a TGA codon at nucleotides 1044-1046. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 1D, and the start and stop codons are in bold letters.

Table 1D. GPCR1b Nucleic acid sequence (SEQ ID NO:3).

TCGCCATGTACAACGGGTCGTGCTGCCGCATCGAGGGGACACCATCTCCAGGTGATGCCGCCGCTGCTCATTGTGG
CCTTTGTGCTGGGCGCACTAGGCAATGGGGTCGCCCTGTGTGGTTCTGCTTCCACATGAAGACCTGGAAGCCCAGCA
CTGTTTACCTTTTCAATTTGGCCGTGGCTGATTTCCCTCCTTATGATCTGCCTGCCTTTTCGGACAGACTATTACCTCA
GACGTAGACACTGGGCTTTTGGGGACATTCCCTGCCGAGTGGGGCTCTTCACGTTGGCCATGAACAGGGCCGGGAGCA
TCGTGTTCTTACGGTGGTGGCTGCGGACAGGTATTTCAAAGTGGTCCACCCCCACCACGCGGTGAACACTATCTCCA
CCCGGGTGGCGGCTGGCATCGTCTGCACCCCTGTGGGCCCTGGTCATCCTGGGAACAGTGTATCTTTTGTGGAGAACC
ATCTCTGCGTGCAAGAGACGGCCGTCTCCTGTGAGAGCTTCATCATGGAGTCGGCCAATGGCTGGCATGACATCATGT
TCCAGCTGGAGTTCCTTATGCCCTCGGCATCATCTTATTTTGTCTCCTTCAAGATTGTTTGGAGCCTGAGGCGGAGGC
AGCAGCTGGCCAGACAGGCTCGGATGAAGAAGGCGACCCGGTTCATCATGGTGGTGGCAATTGTGTTTCATCACATGCT
ACCTGCCACGCGTGTCTGCTAGACTCTATTTCTCTGGACGGTGCCTCGAGTGCCTGCGATCCCTCTGTCCATGGGG
CCCTGCACATAACCTCAGCTTCACCTACATGAACAGCATGCTGGATCCCTGGTGTATTATTTTCAAGCCCCCTCT
TTCCCAAATTCTACAACAAGCTCAAAATCTGCAGTCTGAAACCCAAGCAGCCAGGACACTCAAAAACACAAAGGCCGG
AAGAGATGCCAATTTCAACCTCGGTGCGAGGAGTTGCATCAGTGTGGCAAATAGTTTCAAAGCCAGTCTGATGGGC
AATGGGATCCCCACATTGTTGAGTGGCACTGAACAA

The encoded protein is the same as for GPCR1a and is disclosed above in Table 1B.

GPCR1c

A disclosed GPCR1c (also referred to as AC011711_da1) nucleic acid of 1104 nucleotides is shown in Table 1E. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 60-62 and ending with a TGA codon at nucleotides 1098-1100. Putative untranslated regions 5' to the start codon and 3' to the stop codon are underlined in Table 1E and the start and stop codons are in bold letters.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 530 of 770 bases (68%) identical to a gb:GENBANK-ID:HUMHM74|acc:D10923.1 mRNA from *Homo sapiens* (Human mRNA for HM74).

Table 1E. GPCR1c Nucleic acid sequence (SEQ ID NO:4).

GTGCCATTGTGGGGAATCCCTGGGCTGCTCTGCACCCGGACACTTGCTCTGTCCCGCCATGTACAACG
GGTCTGTGCTGCCGCATCGAGGGGGACACCATCTCCAGGTGATGCCGCCGCTGCTCATTGTGGCCTTTG
TGCTGGGCGCACTAGACAATGGGGTCGCCCTGTGTGGTTTCTGCTTCCACATGAAGACCTGGAAGCCCA
GCACTGTTTACCTTTTCAATTTGGCCGTGGCTGATTTCCCTCCTTATGATCTGCCTGCCTTTTCGGACAGA
CTATTACCTCAGACGTAGACACTGGGCTTTTGGGGACATTCCCTGCCGAGTGGGGCTCTTCACGTTGGC
CATGAACAGGGCCGGGAGCATCGTGTTCCTTACGGTGGTGGCTGCGGGCAGGTATTTCAAAGTGGTCCA
CCCCACCACGCGGTGAACACTATCTCCACCCGGTGGCGGCTGGCATCGTCTGCACCCCTGTGGGCCCT
GGTCATCCTGGGAACAGTGTATCTTTTGTGGAGAACCATCTCTGCGTGCAAGAGACGGCCGTCTCCTG
TGAGAGCTTCATCATGGAGTCGGCCAATGGCTGGCATGACATCATGTTCCAGCTGGAGTTCTTTATGCC
CCTCGGCATCATCTTATTTTGTCTCCTTCAAGATTGTTTGGAGCCTGAGGCGGAGGCAGCAGCTGGCCAG
ACAGGCTCGGATGAAGAAGGCGACCCGGTTCATCATGGTGGTGGCAATTGTGTTTCATCACATGCTACCT
GCCAGCGTGTCTGCTAGACTTATTTCTCTGACGGTGCCTCGAGTGCCTGCGATCCCTCTGTCCA
TGGGGCCCTGCACATAACCTCAGCTTCACCTACATGAACAGCATGCTGGATCCCTGGTGTATTATTTT
TCAAGCCCCCTCTTTCCCAAATTCTACAACAAGCTCAAAATCTGCAGTCTGAAACCCAAGCAGCCAGGAC
ACTCAAAAACACAAAGGCCGGAAGAGATGCCAATTTCAACCTCGGTGCGAGGAGTTGCATCAGTGTGG
CAAATAGTTTCAAAGCCAGTCTGATGGGCAATGGGATCCCCACATTGTTGAGTGGCACTGAACAA

The disclosed GPCR1c protein having 346 amino acid residues is presented using the

one-letter code in Table 1F. An analysis using the PSORT program predicts that the AC011711_dal protein localizes in the plasma membrane with a certainty=0.6400. It is also predicted that protein has a signal peptide whose most likely cleavage site is between residues 36 and 37: GVA-LC in Table 1F.

5

Table 1F. Encoded GPCR1c protein sequence (SEQ ID NO:5).

MYNGSCCRIEGDTISQVMPPLLI VAFVLGALDNGVALCGFCFHMKTWKPSTVYLFNLAVADFLLMICLPFR
TDYYLRRRHWAFGDIPCRVGLFTLAMNRAGSIVFLTVVAAGRYFKVVHPHNAVNTISTRVAAGIVCTLWAL
VILGTVYLLLENHLCVQETAVSCSFIMESANGWHDIMFQLEFFMPLGIILFCSFKIVWSLRRRQQLARQA
RMKKATRFIMVVAIVFITCYLPSVSARLYFLWTVPSACDPSVHGALHITLSFTYMNSMLDPLVYYFSSPS
FPKFYNKLKICSLKPKQPGHSKTQRPEEMPISNLGRRSCISVANSFQSQSDGQWDPHIVEWH

A BLASTX search was performed against public protein databases. The full amino acid sequence of the protein of the invention was found to have 270 of 317 amino acid residues 176 of 339 amino acid residues (51%) identical to, and 225 of 339 amino acid residues (66%) similar to, the 387 amino acid residue ptmr:SWISSPROT-ACC:P49019 protein from *Homo sapiens* (Human) (PROBABLE G PROTEIN-COUPLED RECEPTOR HM74). In the following positions, one or more consensus positions of the nucleotide sequence have been identified as single nucleotide polymorphisms (SNPs). "Depth" represents the number of clones covering the region of the SNP. The Putative Allele Frequency (PAF) is the fraction of all the clones containing the SNP. The sign ">" means "is changed to". :Possible SNPs found for GPCR1c are listed in Table 1G.

Table 1G: SNPs

Consensus Position	Depth	Base Change	PAF
334	71	T>C	0.028
677	92	T>C	0.022
719	79	T>C	0.025

The amino acids differences between the three GPCR1 proteins are shown in Table 1H. Deletions are marked by a delta (Δ). The differences between the three proteins appear to be localized to a few distinct regions. Thus, these proteins may have similar functions, such as serving as olfactory or chemokine receptors (see below).

Table 1H. Differences for GPCR1 Proteins


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gi|464327| HVNLYGSTIFLTCISVHRYTGVVHPLKSLGRKKKNNAVYSSSLVWALVVAIAPILFYSG
gi|497261| HVNLYGSTIFLTCISVHRYTGVVHPLKSLGRKKKNNAVYSSSLVWALVVAIAPILFYSG

5      190      200      210      220      230      240
GPCR1a  ....|....|....|....|....|....|....|....|....|....|....|
GPCR1c  LCVQ-ETAVSCBSFIMESANG---WHHIMFQLEFFFMPLGIIILFCSFKIYWSLRQRQ--I
gi|2135386| LCVQ-ETAVSCBSFIMESANG---WHHIMFQLEFFFMPLGIIILFCSFKIYWSLRQRQ--I
gi|11558404| LIQN-GPANVCISFSICHTFR---WHHIMFQLEFFFMPLGIIILFCSARIWWSLRQRQ--M
gi|6716509| MTKN-GEAYLCSSFSICYNFR---WHHIMFQLEFFFMPLGIIILFCSGRIWWSLRQRQ--M
gi|464327| TTQN---TECPSEFYPTGGTKAIATCQVLEFLOVLLPFGLISFCNSGRIWWSLRQRQ--M
gi|497261| TGVRNRKTITCYDTTADEYLRSYFVYSMCTTVFMCIPIFIVILGCYGLIVKALIYKD--Y

10     250     260     270     280     290     300
GPCR1a  ARQARMKKAIRFIMVVAIVEITCMLPSVSARHYFLWTVPSACD-----PSVHGALHET
GPCR1c  ARQARMKKAIRFIMVVAIVEITCMLPSVSARHYFLWTVPSACD-----PSVHGALHET
gi|2135386| DRHAKIKRAITFIMVVAIVEITCMLPSVVRIRIFWLLHTSGTQNCVEY-RSVDLAFFIT
gi|11558404| DRHAKIKRAITFIMVVAIVEITCMLPSVAVRIRIFWLLKYNVNRNDIY-SSVDLAFIT
gi|6716509| DKQPTIRRRARLVANMLLEFGCLPSVLTRVLYHIFQEFKSCS---VQQAIRASDTA
gi|464327| DNSPLRRKSIYLVIIILTVFAVSLPHFVMTLNLRLARLDFQTPQMCAFNKVVATYQVT
gi|497261| DNSPLRRKSIYLVIIILTVFAVSLPHFVMTLNLRLARLDFQTPQMCAFNKVVATYQVT

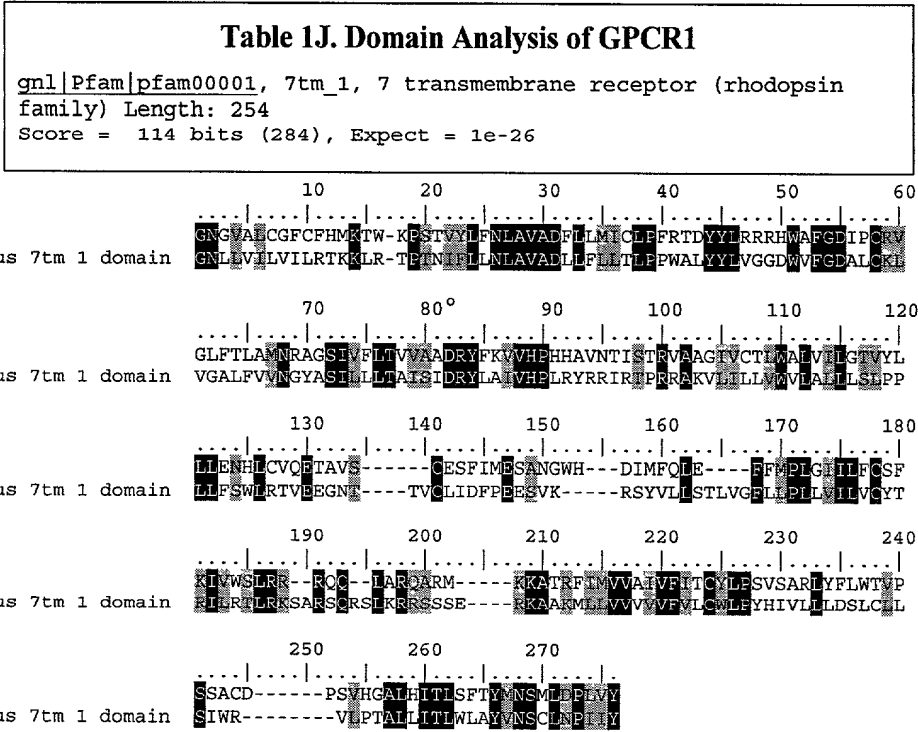
15     310     320     330     340     350     360
GPCR1a  LSFYYMNSMLDPVYVYFSSPSFPKFKYNLKICSLKPKQPGHSKTORPEEMPISNLGRRSC
GPCR1c  LSFYYMNSMLDPVYVYFSSPSFPKFKYNLKICSLKPKQPGHSKTORPEEMPISNLGRRSC
gi|2135386| LSFYYMNSMLDPVYVYFSSPSFPNFTTLINRCLQKMTGEPDNNRSTSVELTGDPNK-T
gi|11558404| LSFYYMNSMLDPVYVYFSSPSFPNFTTCINRCLRKKTLGEPDNNRSTSVELTGDPST-T
gi|6716509| GSLICLHSTLSPATYCESNPAFTHSYRKLKSLRGRR-----KAAESPSD-----
gi|464327| RGLASLNSCYDPILYLAGDTFRRLSRATRKSSRRS---EP-NVQSKSEMT-----
gi|497261| RGLASLNSCYDPILYLAGDTFRRLSRATRKSSRRS---EP-NVQSKSEMT-----

20     370     380     390     400
GPCR1a  ISVANSTQSQSDGQWDPHIVEWH-----
GPCR1c  ISVANSTQSQSDGQWDPHIVEWH-----
gi|2135386| RGAPFALMANSGEPSYLGPTSNNHSSKKGHCHEPASLEKQLGCCIE
gi|11558404| RSLPGALMADPSEPGSPPYLASTSR-----
gi|6716509| -NLRDSYS-----
gi|464327| LNLITEXQN---GDTSL-----
gi|497261| LNLITEXQN---GDTSL-----

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The presence of identifiable domains in GPCR1, as well as all other GPCR proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro>). DOMAIN results, *e.g.*, for GPCR1 as disclosed in Table 1J, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. For Table 1J and all successive DOMAIN sequence alignments, fully conserved single residues are indicated by black shading and “strong” semi-conserved residues are indicated by grey shading. The “strong” group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 1J lists the domain description from DOMAIN analysis results against GPCR1. The region from amino acid residue 53 through 239 (SEQ ID NO:2) most probably ($E = 2e^{-19}$) contains a “seven transmembrane receptor (rhodopsin family) fragment” domain, aligned here with residues 12-180 of the 7tm_1 entry (TM7, SEQ ID NO:45, see Table 1K for the complete sequence) of the Pfam database. This indicates that the GPCR1 sequence has properties similar to those of other proteins known to contain this domain as well as to the 377 amino acid 7tm domain itself.



The representative member of the 7 transmembrane receptor family is the D2 dopamine receptor from *Bos taurus* (SWISSPROT: locus D2DR_BOVIN, accession P20288; gene index 118205). The D2 receptor is an integral membrane protein and belongs to Family 1 of G-protein coupled receptors. The activity of the D2 receptor is mediated by G proteins which inhibit adenylyl cyclase. Chio *et al.*, Nature 343:255-269 (1990). Residues 51-427 of this 444 amino acid protein are considered to be the representative TM7 domain, shown in Table 1Q.

Table 1Q Amino Acid sequence for TM7 (SEQ ID NO:45)

GNVLVCMASREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF
VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVVWLSFTISCPM
LFGLNNTDQNECIIANPAFVVYSSIVSFYVFPFIVTLLVYIKIYIVLRRRRKRVNTRKSSR
AFRANLKAPLKGNCTHPEDMKLCTVIMKSNFSFPVNRVRVEAARRAQELEMELSSSTSP
ERTRYSPIPPSSHQLTLPDPSHHGLHSTPDSPAKPEKNGHAKTVNPKIAKIFEIQSMPNG
KTRTSLKTMSRRKLSQQKEKKATQMLAIVLGVFIICWLPFFITHILNIHCDNIPPVLYS
AFTWLGYN SAVNP I I Y

The 7 transmembrane receptor family includes a number of different proteins, including, for example, serotonin receptors, dopamine receptors, histamine receptors, andrenergic receptors, cannabinoid receptors, angiotensin II receptors, chemokine receptors, opioid receptors, G-protein coupled receptor (GPCR) proteins, olfactory receptors (OR), and the like. Some proteins and the Protein Data Base Ids/gene indexes include, for example: rhodopsin (129209); 5-hydroxytryptamine receptors; (112821, 8488960, 112805, 231454, 1168221, 398971, 112806); G protein-coupled receptors (119130, 543823, 1730143, 132206, 137159, 6136153, 416926, 1169881, 136882, 134079); gustatory receptors (544463, 462208); c-x-c chemokine receptors (416718, 128999, 416802, 548703, 1352335); opsins (129193, 129197, 129203); and olfactory receptor-like proteins (129091, 1171893, 400672, 548417);

GPCR1 is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to proprietary database sources, Public EST sources, Literature sources, and/or RACE sources.

The nucleic acids and proteins of GPCR1 are useful in potential therapeutic applications implicated in various GPCR- or olfactory receptor (OR)-related pathologies and/or disorders. For example, a cDNA encoding the G-protein coupled receptor-like protein

may be useful in gene therapy, and the G-protein coupled receptor-like protein may be useful when administered to a subject in need thereof. The novel nucleic acid encoding GPCR1 protein, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. The GPCRX nucleic acids and proteins are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: developmental diseases, MHCII and III diseases (immune diseases), Taste and scent detectability Disorders, Burkitt's lymphoma, Corticoneurogenic disease, Signal Transduction pathway disorders, Retinal diseases including those involving photoreception, Cell Growth rate disorders; Cell Shape disorders, Feeding disorders; control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidoluysian atrophy(DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Osteodystrophy. Additional GPCR-related diseases and disorders are mentioned throughout the Specification.

Further, the protein similarity information, expression pattern, and map location for GPCR1 suggests that GPCR1 may have important structural and/or physiological functions characteristic of the GPCR family. The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for

potential agonist and antagonist compounds. For example, a cDNA encoding GPCR1 may be useful in gene therapy, and GPCR1 may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding OR-like protein, and the OR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR1 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR_X Antibodies" section below. The disclosed GPCR1 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR1 epitope is from about amino acids 1 to 10. In another embodiment, a GPCR1 epitope is from about amino acids 75 to 100. In additional embodiments, GPCR1 epitopes are from amino acids 130 to 140, 210-230, and from amino acids 270 to 330. These novel proteins can also be used to develop assay system for functional analysis.

GPCR2

An additional GPCR-like protein of the invention, referred to herein as GPCR2, is an Olfactory Receptor ("OR")-like protein. The novel nucleic acid of 1149 nucleotides (80250319_EXT, SEQ ID NO:6) encoding a novel G-protein coupled receptor-like protein is shown in Table 2A.

Table 2A. GPCR2 Nucleotide Sequence (SEQ ID NO:6)

ATGCCCGATGCAGCCACGATAGCCACCATGAATAAGGCAGCAGGCGGGGACAAGCTAGCAGAACTCTTCAGTCTGGT
CCCCGACCTTCTGGAGGCGGCCAACACGAGTGGTAACGCGTCGCTGCAGCTTCCGGACTTGTGGTGGGAGCTGGGGC
TGGAGTTGCCGGACGGCGCGCCGAGGACATCCCCCGGGCAGCGGCGGGGACAGAGCGCGGACACAGAGGCCCGG
GTGCGGATTCTCATCAGCGTGGTGTACTGGGTGGTGTGCGCCCTGGGGTTGGCGGGCAACCTGCTGGTTCTCTACCT
GATGAAGAGCATGCAGGGCTGGCGCAAGTCCTCTATCAACCTCTTCGTCACCAACCTGGCGCTGACGGACTTTCAGT
TTGTGCTCACCCCTGCCCTTCTGGGCGGTGGAGAAGCTCTTGACTTCAAATGGCCCTTCGGCAAGGCCATGTGTAAG
ATCGTGTCCATGGTGACGTCATGAACATGTACGCCAGCGTGTCTTCTCACTGCCATGAGTGTGACGCGCTACCA
TTCGGTGGCCTCGGCTCTGAAGAGCCACCGACCCGAGGACACGCGCGGGGCGACTGCTGCGGCCGGAGCCTGGGGG
ACAGCTGCTGCTTCTCGGCCAAGCGCTGTGTGTGGATCTGGGCTTTGGCCGCGCTGGCTCGCTGCCAGTGCC
ATTTTCTCCACCACGGTCAAGGTGATGGGCGAGGAGCTGTGCACTGGTGCCTTTCCGGACAAGTTGCTGGGCGCG
ACAGGCAGTTCTGGCTGGGCTCTACCACTCGCAGAAGAAGCTGCTGGGGTACCGGCTTACTTAGCATATATTTTAA
TTCCAAAACAATTCTTTAGATCACTACCTCTTCTTACGACCTCTGTATTTCCGCCCTCTCTTACCTTCCGTT
ATCCGCAACATTTCTCTCTTACCGCCACAACACGATAAACCGCGTAGGACCTGGTGTCCACCCCATGGACTGGACC
CGCCAGTCCAGACCAGATTGAAAATACGTATAGATTGCTACCTGCTATGTACATCACTATGAATTTCTGGCATTTA
AATCAAAACAGATTTTCAGGAAGTACCTGGGACTCAGACACCATTAAACCTTGGGAAAGCATGTTTTGA

An open reading frame (ORF) for GPCR2 was identified from nucleotides 1 to 1146. The disclosed GPCR2 polypeptide (SEQ ID NO:7) encoded by SEQ ID NO:6 is 382 amino acid residues and is presented using the one-letter code in Table 2B. The GPCR2 protein was analyzed for signal peptide prediction and cellular localization. SignalPep results predict that GPCR2 is cleaved between position 33 and 34 of SEQ ID NO:8, *i.e.*, at EAA-NT. Psort and Hydropathy profiles also predict that GPCR2 contains a signal peptide and is likely to be localized at the plasma membrane (certainty of 0.6000).

Table 2B. Encoded GPCR2 protein sequence (SEQ ID NO:7).

MADAATIATMKAAGGDKLAELFSLVLDLLEANTSGNASLQLPDLWWELGLELPDGAPPGHPPGSGGAESADTEAR
VRILISVVYVWVVCALGLAGNLLVLYLMKSMQGWKSSINLFVTNLALTDQFVLTLPFWAVENALDFKWPFGKAMCK
IVSMVTSNMNYASVFLTAMSVTRYHSVASALKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIWALAALASLP
IFSTTVKVMGEELCTGAFFGQVAGPRQAVLAGPLPLAEAAAGVPAYLAYIFIPKQFFRSLPLSYDLLYFPPLSYPSV
IRNISSLPPQHDKPRRTWCPPPWTPGASPDQIENTYRFATCYVHHYEFLLAFKSNRFSGLTQTPFKPWESMF

The GPCR2 nucleic acid sequence has 405 of 648 nucleotides (62%) identical to Sequence 9 from patent US 5436155 (GENBANK-ID:I13406)

The full amino acid sequence of the protein of the invention was found to have 62 of 170 amino acid residues (36%) identical to, and 96 of 170 amino acid residues (56%) positive with, the 359 amino acid residue protein from *Rattus norvegicus* (SWISSPROT-ACC:P29089). The protein encoded by GPCR2 (SEQ ID NO:6) has significant homology to olfactory, odorant, and taste chemoreceptors and belongs to the family of G-Protein coupled receptors (GPCRs). This family of genes has been used as a target for small molecule drugs

and GPCRs are expressed on the plasma membrane and are also a suitable target for protein drugs like therapeutic antibodies, cytotoxic antibodies and diagnostic antibodies.

Another BLAST against GenBank Accession Number: XP_003874.1, a 471 amino acid G-protein coupled receptor SALPR; somatostatin and angiotensin-like peptide receptor protein from *Homo sapiens*, produced 100% identity, between a 245 fragment and amino acids 1-247 of GPCR2 (Table 2C).

Table 2C: Alignment of GPCR2 with gi 11416964 ref XP_003874.1 G-protein coupled receptor SALPR; somatostatin and angiotensin-like peptide receptor [Homo sapiens] (SEQ ID NO:86)	
Score = 396 bits (1018), Expect = e-109	
Identities = 207/255 (81%), Positives = 209/255 (81%)	
<div><div>102030405060</div><div>GPCR2</div><div>gi 11416964 </div><div>-----MADAATATATMNKAAGGDKLAELFSLVDPDLLEAANTSGNASLQLPDLWWELGLELPDGA</div></div>	
<div><div>708090100110120</div><div>GPCR2</div><div>gi 11416964 </div><div>PPGHPPGSGGAESADTEARVRILISVVYVWVVCALGLAGNLLVLYLMKSMQGWRKSSINLF</div></div>	
<div><div>130140150160170180</div><div>GPCR2</div><div>gi 11416964 </div><div>VTNLALTDFQFVLTLPFWAVENALDFKWPFKAMCKIVSMVTSMMMYASVFFLTAMSVTR</div></div>	
<div><div>190200210220230240</div><div>GPCR2</div><div>gi 11416964 </div><div>YHSVASALKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIWALAALASLPSAIFSTTVK</div></div>	
<div><div>250260270280290300</div><div>GPCR2</div><div>gi 11416964 </div><div>VMGEELCTGAFFGQVAG-----PRQAVLAGPLPL</div></div>	
<div><div>310320330340350360</div><div>GPCR2</div><div>gi 11416964 </div><div>-----AEEAAG--VPAYLAYTFIPKQFFRSIPLSYDILYFE</div></div>	
<div><div>370380390400410420</div><div>GPCR2</div><div>gi 11416964 </div><div>YPSVIRNISSLEPQHDKPRRTWCPPWTGPASPDQIENTYRFATCYVHH--YEFLAPK--</div></div>	
<div><div>430440450460470</div><div>GPCR2</div><div>gi 11416964 </div><div>-----SNRFGTSLGTCSTEFKEWESMF</div></div>	

Other BLAST results including the sequences used for ClustalW analysis are presented in Table 2D.

Table 2D. BLAST results for GPCR2

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 12188901 emb CAC21550.1 (AJ301623)	angiotensin II type 1 receptor [Cavia porcellus]	359	46/109 (42%)	61/109 (55%)	6e-16
>gi 8927995 sp Q9WV26 AG2R_CAVPO	TYPE-1 ANGIOTENSIN II RECEPTOR (AT1) [Cavia porcellus]	359	45/109 (41%)	61/109 (55%)	1e-15
gi 90297 pir JH0621	angiotensin II receptor 1A - mouse	359	44/109 (40%)	61/109 (55%)	2e-15
gi 249946 gb AAB22269.1 (S37484)	Angiotensin II receptor isoform 1a, AT-1a receptor Balb/c, liver	359	44/109 (40%)	61/109 (55%)	2e-15

This information is presented graphically in the multiple sequence alignment given in Table 2E (with GPCR2a being shown on line 1) as a ClustalW analysis comparing GPCR2a with related protein sequences.

Table 2E. Information for the ClustalW proteins:

- 1) Novel GPCR2a (SEQ ID NO:8)
- 2) gi|11416964|ref|XP_003874.1| G-protein coupled receptor SALPR; somatostatin and angiotensin-like peptide receptor [Homo sapiens]
- 3) >gi|12188901|emb|CAC21550.1| (AJ301623) angiotensin II type 1 receptor [Cavia porcellus]
- 4) gi|8927995|sp|Q9WV26|AG2R_CAVPO TYPE-1 ANGIOTENSIN II RECEPTOR (AT1)
- 5) >>gi|90297|pir|JH0621 angiotensin II receptor 1A - mouse
- 6) >gi|249946|gb|AAB22269.1| (S37484) angiotensin II receptor isoform 1a, AT-1a receptor

	10	20	30	40	50	60
GPCR2
gi 11416964
gi 12188901
gi 8927995
gi 90297
gi 249946
	70	80	90	100	110	120
GPCR2
gi 11416964
gi 12188901
gi 8927995
gi 90297
gi 249946
	130	140	150	160	170	180
GPCR2
gi 11416964
gi 12188901
gi 8927995
gi 90297
gi 249946

gi 249946	LLNLALADLCFLLTLPWAVYTAMEYRWPFGRNHCCKIASASVSFNLYASVFLLTCLSLDR
	190 200 210 220 230 240
GPCR2	YHSVASATKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIIWALALASLPSAIFSTTVK
gi 11416964	YHSVASATKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIIWALALASLPSAIFSTTVK
gi 12188901	YLAIVHPMKSLRRTM-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF
gi 8927995	YLAIVHPMKSLRRTM-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF
gi 90297	YLAIVHPMKSLRRTM-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF
gi 249946	YLAIVHPMKSLRRTM-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF
	250 260 270 280 290 300
GPCR2	VMGEELCTGAFFPGQVAG-----PRQAVTAGPLEL-----
gi 11416964	VMGEELCTVRFDPKLLGRDQFNLGLYHSCQVLELGFVLPGLIILLCYLLLVRFIADRRAA
gi 12188901	IENTNITVCAFYHESNSTLPIGLG---LTKNILGFPPFLIILTSY-----
gi 8927995	IENTNITVCAFYHESNSTLPIGLG---LTKNILGFPPFLIILTSY-----
gi 90297	IENTNITVCAFYHESNSTLPIGLG---LTKNILGFPPFLIILTSY-----
gi 249946	IENTNITVCAFYHESNSTLPIGLG---LTKNILGFPPFLIILTSY-----
	310 320 330 340 350 360
GPCR2	-----AEEAAG--VPAYLAYIFIPKQFFRSIPLSYDLLVFE-----PLS
gi 11416964	GTKGGA--MAGGRPTGASARELSKVTKSTINVLSEFLQWEPICALITWSLILKFNAMPFS
gi 12188901	-TLIWKALKKAYEIQKNKPRNDDIFRIIMAIVLFFFFSWVPHQIFFLDVLQIQLG-VIHD
gi 8927995	-TLIWKALKKAYEIQKNKPRNDDIFRIIMAIVLFFFFSWVPHQIFFLDVLQIQLG-VIHD
gi 90297	-TLIWKALKKAYEIQKNKPRNDDIFRIIMAIVLFFFFSWVPHQIFFLDVLQIQLG-VIHD
gi 249946	-TLIWKALKKAYEIQKNKPRNDDIFRIIMAIVLFFFFSWVPHQIFFLDVLQIQLG-VIHD
	370 380 390 400 410 420
GPCR2	YPSVIRNISSEPPQHDKPRRTWCPPWTGPASPDQIENTYRFATCVHHH--YEFLAFK--
gi 11416964	QBYFLCOVYAFPPSYCLAHNSCLNPLVLYCLVRRFFFKALKSLIWRHAS--PSLTSMRPF
gi 12188901	CKISDIVDTAMPITICIAFYNNCLNPLFYGLGKKFKKYFLQLLKYIPPAKSHSLSTK
gi 8927995	CKISDIVDTAMPITICIAFYNNCLNPLFYGLGKKFKKYFLQLLKYIPPAKSHSLSTK
gi 90297	CKISDIVDTAMPITICIAFYNNCLNPLFYGLGKKFKKYFLQLLKYIPPAKSHSLSTK
gi 249946	CKISDIVDTAMPITICIAFYNNCLNPLFYGLGKKFKKYFLQLLKYIPPAKSHSLSTK
	430 440 450 460 470
GPCR2	-----SNRSGISLGIQTPFKFWESMF-----
gi 11416964	TAITKPEHEQGLQAPAPAHAAEPDLLYYPPGVVYSGGRYDLLPSSSAY
gi 12188901	MSTLSYRPSDNVSSSAKKEVQCSEVE-----
gi 8927995	MSTLSYRPSDNVSSSAKKEVQCSEVE-----
gi 90297	MSTLSYRPSDNVSSSAKKEASCSEVE-----
gi 249946	MSTLSYRPSDNVSSSAKKEASCSEVE-----

DOMAIN results for GPCR2 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The 7tm_1, a seven transmembrane receptor (rhodopsin family), was shown to have homology to GPCR2. (E= 8e-20). This indicates that the GPCR2 sequence has properties similar to those of other proteins known to contain this domain as well as to the 7tm_1 domain itself (Table 2F).

Table 2F. Domain Analysis of GPCR1

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) Length: 254
CD-Length = 254 residues, only 50.0% aligned
Score = 91.3 bits (225), Expect = 8e-20

	10 20 30 40 50 60
GPCR2	-NLLVVEYLMKSMQGWK-SSINLFFVTNLATLFCQVLTLPFWAVENALDFKMPFGKAMCK
Consensus 7tm 1 domain	GNLLVVEYLMKSLRRTKLR--LPTNIFELNLAWADLLFELTLPFWAYYLGGDWVFGDALCK

5 GPCR2
Consensus 7tm 1 domain

10 GPCR2
Consensus 7tm 1 domain

15 GPCR2
Consensus 7tm 1 domain

20 GPCR2
Consensus 7tm 1 domain

70 80 90 100 110 120

130 140 150 160 170 180

190 200 210 220 230 240

250 260 270 280 290

IVSMVTSNNMYASVFPLTAMSVTRYHSEVASALKSHRTTGHGEGDCCGRSLGDSCCFSAKA
LVGALFVYNGYASILLTLTASIDRYLAIVHPLRYRRIITPRK-----AKV

LCVWVWALAAASLPSAHSSTTVKVMGEE-----CTGAFEGGVAGP--EQAVLAGPLP
LILLWVVLALDLSLEPLPSWLRITVEEGNT---TVCLIDFEEISVK---RSYVLLSTL-

LAEEAAGVPAYIAYEFIPKOFFRSPLSYDLLYFPPLSYPSVIKNISSPPQHDKPRRTW
---VGFTLPLLVIVCYTRILRLRKSARSQSLKRRSSSERKAAKMLLVVVVVVFLCW

CEPPWTGPASPDQIENTYR-----FATCYVHHYELAFKSNRFSGTSLGTQTPFKP
LPYHIVLLDLSLCLLSIR-----VLPTAILITLQLAVVNSCLNPIIY-----

Based on information available on expression of SWISSPROT-ACC:P29089 TYPE-1B ANGIOTENSIN II RECEPTOR (AT1B) (AT3) - *Rattus norvegicus* (Rat), the closest G-Protein Coupled Receptor family member it is likely that GPCR2 is expressed in cardiac tissue, renal tissue, and vascular tissue as angiotensin is expressed in these tissues.

GPCR2 includes the nucleic acid whose sequence is provided in Table 2A, or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 2A while still encoding a protein that maintains its G-Protein Coupled Receptor-like activities and physiological functions, or a fragment of such a nucleic acid. GPCR2 further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. GPCR2 additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 38 percent of the bases may be so changed.

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor -like protein may be useful in gene therapy, and the olfactory receptor -like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of

patients suffering from Cardiovascular disorders, Hypertension, Diabetes, Autoimmune disease, Renal artery stenosis, Interstitial nephritis, Glomerulonephritis, Polycystic kidney disease, Systemic lupus erythematosus, Renal tubular acidosis, IgA nephropathy, Hypercalcaemia, Lesch-Nyhan syndrome, Cardiomyopathy, Atherosclerosis, Hypertension, Congenital heart defects, Aortic stenosis, Atrial septal defect (ASD), Atrioventricular (A-V) canal defect, Ductus arteriosus, Pulmonary stenosis, Subaortic stenosis, Ventricular septal defect (VSD), valve diseases, Tuberous sclerosis, Scleroderma, Obesity, Cell signalling disorders, Cancer, Muscular degeneration, Metabolic and Endocrine disorders, Respiratory disorders, Tissue/Cell growth regulation disorders, and Developmental disorders. Other GPCR-related diseases and disorders are contemplated.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. The disclosed GPCR2 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR2 epitope is from about amino acids 50 to 90. In another embodiment, a GPCR2 epitope is from about amino acids 180 to 230. In an additional embodiment, GPCR2 epitopes are from amino acids 310 to 360.

These novel proteins can be used in assay systems for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

GPCR3

An additional GPCR-like protein of the invention, referred to herein as GPCR3, is an Olfactory Receptor ("OR")-like protein. The novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probe or homolog, run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The novel nucleic acid of 970 nucleotides (AC020597, SEQ ID

NO:8) encoding a novel olfactory receptor-like protein is shown in Table 3A. An open reading frame (ORF) was identified beginning with an ATG initiation codon at nucleotides 27-29 and ending with a TAA codon at nucleotides 947-949. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 3A, and the start and stop codons are in bold letters.

Table 3A. GPCR3 Nucleotide Sequence (SEQ ID NO:8)

AAAAAGTTCCGAGAAGAACGGCCTCAATGAATACCACTCTATTTTCATCCTTACTCTTTCTTCTTCT
GGGAATTCCTGGGCTGGAAAGTATGCATCTCTGGGTTGGTTTTCTTTCTTTGCTGTGTTCTGAC
AGCTGTCCTTGGGAATATCACCATCCTTTTTGTGATTGAGACTGACAGTAGTCTCCATCATCCCAT
GTTCTACTTCTGGCCATTCTGTCATCTATTGACCCGGGCCTGTCTACATCCACCATCCCTAAAATG
CTTGGCACCTTCTGGTTTACCCTGAGAGAAATCTCCTTTGAAGGATGCCTTACCCAGATGTTCTTC
ATCCACCTGTGCACTGGCATGGAATCAGCTGTGCTTGTGGCCATGGCCTATGATTGCTATGTGGCC
ATCTGTGACCCTCTTTGCTACACGTTGGTGCTGACAAACAAGGTGGTGTCAGTTATGGCACTGGCC
ATCTTTCTGAGACCCTTAGTCTTTGTCATACCCTTTGTTCTATTTATCCTAAGGCTTCCATTTTGTG
GACACCAAATTATTCCTCATACTTATGGTGAGCACATGGGCATTGCCCCTGTCTTGTGCCAGCA
TCAGGGTTAATCATCTATGGCTTATGTGCCATCTCTATCCTGGTCTTTGACATCATAGCAATTG
TCATTTCTATGTACAGATCCTTTGTGCTGTATTTCTACTCTTTCACATGATGCACGACTCAAGGC
ATTCAGCACCTGTGGCTCTCATGTGTGTGTCATGTTGACTTTCTATATGCCTGCATTTTCTCATT
ATGACCCATAGGTTTGGTCGGAATATACCTCACTTTATCCACATTCTTCTGGTAATTTCTATGTAG
TCATTCCACCTGCTCTCACTCTGTAATTTATGGTGTCAGAACCAACAGATTAGAGCACAAGTGC
TGAAAAATGTTTTCAATAAATAAACATAGCTCATTATA

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of GPCR3 has 627 of 904 bases (69%) identical to a *Mus musculus* odorant receptor S46 gene (GENBANK-ID: AF121979).

The disclosed GPCR3 polypeptide (SEQ ID NO:9) encoded by SEQ ID NO:9 is 308 amino acid residues and is presented using the one-letter code in Table 3B. The GPCR3 protein were analyzed for signal peptide prediction and cellular localization. SignalP results predict that GPCR3 is cleaved between position 40 and 41 of SEQ ID NO:10, *i.e.*, at the slash in the amino acid sequence VLG-NI. Psort and Hydropathy profiles also predict that GPCR3 contains a signal peptide and is likely to be localized at the plasma membrane (certainty of 0.6400).

Table 3B. Encoded GPCR3 protein sequence (SEQ ID NO:9).

MNTTLFHPYSFLLLGIPLGLESMLWVGFFFAVFLTAVLGNITILFVIQTDSSLHHPMFYFLAILSSIDPGLSTS
TIPKMLGTFWFTLREISFEGCLTQMFFIHLCTGMESAVLVAMAYDCYVAICDPLCYTLVLTNKVVSVMALAIFLR
PLVFVIPFVLFILRLPFCGHQIIPHTYGEHMGIA RLSCASIRVNIYGLCAISILVFDIIAIVISYVQILCAVFL
LSSHDARLKAFSTCGSHVCVMLTFYMPAFFSFMTHRFRGNIPHFHILLANFYVVIIPALNSVIYGVRTKQIRAQ
VLKMFENK

		130	140	150	160	170	180
5	GPCR3	VTFFVLEI--LRLPFCGH-----QIIPTYGEHMGTLRLSCAET--VNIIYG					
	Consensus 7tm 1 domain	SLFPLLES-WLRTVEEGNT-----TVCHIDFPEESVK--RSYVL					
		190	200	210	220	230	240
10	GPCR3	CAISILVFD--AIIVSYVGLIC-----					
	Consensus 7tm 1 domain	STLVGFLLP--LVVLCYTRILR-----					
		250	260	270	280	290	300
15	GPCR3						
	Consensus 7tm 1 domain						
		310	320	330	340	350	360
20	GPCR3	---AVFLSSHDAR--LKAFSTCGS-----HVOVMITF--YMEAFFSFMTHRFGRN--					
	Consensus 7tm 1 domain	---TLRKSARSQSLEKRRSSERKAAKMLLVVVVFVLCWLPYHIVLLDLSLCLLSIWR					
		370	380	390			
25	GPCR3	---EHPFTEHLLANFYVVPALNSVIY					
	Consensus 7tm 1 domain	---VETAILLTWLAVNS--CLNPIIV					

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor -like protein may be useful in gene therapy, and the olfactory receptor -like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from infections such as bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. Other GPCR-related diseases and disorders are contemplated.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These novel proteins can be used in assay systems for functional analysis of various human disorders, which will help in

understanding of pathology of the disease and development of new drug targets for various disorders.

GPCR4

GPCR4 includes a family of three nucleic acids disclosed below. The disclosed nucleic acids encode a GPCR-like protein.

GPCR4a

The disclosed GPCR4a (also referred to herein as AC020597_B) is encoded by a nucleic acid, 994 nucleotides long (SEQ ID NO:10). An open reading frame was identified beginning with an ATG initiation codon at nucleotides 23-25 and ending with a TAA codon at nucleotides 968-970. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4A, and the start and stop codons are in bold letters. The encoded protein having 315 amino acid residues is presented using the one-letter code in Table 4B (SEQ ID NO:11).

Table 4A. GPCR4a Nucleotide Sequence (SEQ ID NO:10).

TGCTGAATTACTCAAAGTCACT**AT**GGGAGACTGGAATAACAGTGATGCTGTGGAGCCCATATTTAT
CCTGAGGGGTTTTCTGGACTGGAGTATGTTCACTTCTGGCTCTCCATCCTCTTCTGTCTTGATA
TTTGGTAGCATTTATGGGTAATGTTACCATCCTGTCTGTCACTTGGATAGAATCCTCTCTCCATCA
GCCCATGTATTACTTTATTTCCATCTTAGCAGTGAATGACCTGGGGATGTCCCTGTCTACACTTCC
CACCATGCTTGCTGTGTTATGGTTGGATGCTCCAGAGATCCAGGCAAGTGCTTGCTATGCTCAGCT
GTTCTTCATCCACACATTACATTCTGGAGTCCTCAGTGTTGCTGGCCATGGCCTTTGACCGTTTT
GTTGCTATCTGCCATCCACTGCACTACCCACCATCCTCACCAACAGTGTAATTGGCAAATTGGTT
TGGCCTGTTTGCTACGAAGCTTGGGAGTTGTACTTCCACACCTTTGCTACTGAGACACTATCACTA
CTGCCATGGCAATGCCCTCTCTCAGCCTTCTGTTGCAACAGGATGTTCTAAGATTATCCTGTACA
GATGCCAGGACCAACAGTATTTATGGGCTTTGTGTAGTCATTGCCACACTAGGTGTGGATTCAATCT
TCATACTTCTTTCTTATGTTCTGATTCTTAATACTGTGCTGGATATTGCATCTCGTGAAGAGCAGCT
AAAGGCACTCAACACATGTGTATCCCATATCTGTGTGGTGCTTATCTTCTTTGTGCCAGTTATTGGG
GTGTCAATGGTCCATCGCTTTGGGAAGCATCTGTCTCCCATAGTCCACATCCTCATGGCAGACATCT
ACCTTCTTCTTCCCCAGTCCTTAACCTATTGTCTATAGTGTGAGAACAAAGCAGATTCGTCTAGG
AATTCCTCACAAAGTTTGTCTTAAGGAGGAGGTTT**AAG**TAACTCTGTCTCCAACCTTTTC

The disclosed nucleic acid GPCR4a sequence has 571 of 868 bases (65%) identical to a *Rattus norvegicus* GPCR mRNA (GENBANK-ID: AF079864).

The GPCR4a polypeptide (SEQ ID NO:11) encoded by SEQ ID NO:10 is presented using the one-letter amino acid code in Table 4B. The Psort profile for GPCR4a predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a GPCR4a peptide is between amino acids 40 and 41, at: LVA-FM., based on the SignalP result.

Table 4B. GPCR4a protein sequence (SEQ ID NO:11)

MGDWNNSDAVEPIFILRGFPGLLEYVHWSLSILFCLAYLVAFMGNVTILSVIWIESSLHQPMMYFISILA
VNDLGMSLSTLPTMLAVLWLDAPFIQASACYAQLFFIHTFTFLESSVLLAMAFDRFVAICHPLHYPTIL
TNSVIGKIGLACLLRSLGVLPPTLLLRHYHYCHGNALSHAFCLHQDVLRLSCTDARTNSIYGLCVVIA
TLGVDSIFILLSYVLILNTVLDIASREEQLKALNTCVSHICVVLIFVFPVIGVSMVHRFGKHLSPIVHI
LMADIYLLLPVLPNPIVYSVRTKQIRLGILHKEVLRRRF

The full amino acid sequence of the disclosed GPCR4a polypeptide has 159 of 301 amino acid residues (52%) identical to, and 217 of 301 residues (72%) positive with, the 319 amino acid residue protein from *Gallus gallus* (ptnr:SPTREMBL-ACC: Q9YH55), ($E = 1.0 \times 10^{-84}$)

BLASTP (Non-Redundant Composite database) analysis of the best hits for alignments with GPCR4a are listed in Table 4C. BLASTX analysis was also performed to determine which proteins have significant identity with GPCR4a, as shown in Table 4D.

Table 4C. BLASTP results for GPCR4a

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ACC:Q9WVN4	MOR 5'BETA1 - Mus musculus	311	160/303 (52%)	219/303 (72%)	1.6e-83
ACC:O88628	PUTATIVE G-PROTEIN COUPLED RECEPTOR RA1C - Rattus norvegicus	320	152/296 (51%)	204/296 (68%)	5.3e-83
ACC:Q9Y5P1	HOR 5'BETA3 - Homo sapiens	312	145/312 (46%)	221/312 (70%)	1.9e-78

Table 4D. BLASTX results for GPCR4a

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P (N)	N
ptnr:SPTREMBL-ACC:Q9YH55 OLFACTORY RECEPTOR-LIKE PROTE.+2		856	1.2e-84	1
ptnr:SPTREMBL-ACC:Q9WVN4 MOR 5'BETA1 - Mus musculus (M.+2		838	9.5e-83	1
ptnr:SPTREMBL-ACC:O88628 PUTATIVE G-PROTEIN COUPLED RE.+2		833	3.2e-82	1
ptnr:SPTREMBL-ACC:Q9WVN5 MOR 5'BETA2 - Mus musculus (M.+2		830	6.7e-82	1
ptnr:SPTREMBL-ACC:Q9WVN6 MOR 5'BETA3 - Mus musculus (M.+2		826	1.8e-81	1
ptnr:SPTREMBL-ACC:Q9Y5P1 HOR 5'BETA3 - Homo sapiens (H.+2		790	1.2e-77	1
ptnr:SPTREMBL-ACC:Q9WU90 ODORANT RECEPTOR S19 - Mus mu.+2		764	6.6e-75	1

Possible SNPs found for GPCR4a are listed in Table 4E.

Table 4E: SNPs

Base Position	Base Before	Base After
115	A	G(4)

140	G	gap(2)
141	C	gap(2)
174	A	gap(17)
175	C	gap(17)
176	A	gap(6)
177	C	gap(6)
178	A	gap(5)
179	C	gap(5)
180	gap	A(22)
181	gap	C(22)
182	gap	A(21)
183	C	gap(20)
185	C	gap(10)
229	gap	A(2)
252	A	gap(2)
256	gap	A(2)
548	T	C(2)
553	G	gap(2)
614	G	A(2)
1445	C	A(2)
1446	C	G(2)
1447	A	G(2)
1448	A	C(2)
1453	G	A(2)
1454	G	T(2)
1455	A	G(2)
1456	C	T(2)
1460	G	C(2)
1462	G	T(2)

GPCR4b

The disclosed GPCR4b (also referred to herein as AC020597B1) is encoded by a nucleic acid, 994 nucleotides long (SEQ ID NO:12). An open reading frame was identified beginning with an ATG initiation codon at nucleotides 23-25 and ending with a TAA codon at nucleotides 968-970. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4F, and the start and stop codons are in bold letters. The encoded protein having 312 amino acid residues is presented using the one-letter code in Table 4D (SEQ ID NO:13).

Table 4F. GPCR4b Nucleotide Sequence (SEQ ID NO:12)

TGCTGAATTACTCAAAGTCACTAT**GGG**GAGACTGGAATAACAGTGATGCTGTGGAGCCCATATTTATC
CTGAGGGGTTTTCTTGGACTGGAGTATGTTCAATTCTGGCTCTCCATCCTCTCTGTCTGCATATT
TGGTAGCATTATGGGTAATGTTACCATCCTGTCTGTCAATTGGATAGAATCCTCTCTCCATCAGCC
CATGTATTACTTTATTTCCATCTTGGCAGTGAATGACCTGGGGATGTCCCTGTCTACACTTCCCACC

ATGCTTGCTGTGTTATGGTTGGATGCTCCAGAGATCCAGGCAAGTGCTTGCTATGCTCAGCTGTTCT
TCATCCACACATTACATTCTGGAGTCCTCAGTGTGCTGGCCATGGCCTTTGACCGTTTTGTTGC
TATCTGCCATCCACTGCACACTACCCACCATCCTCACCAACAGTGTAATTGGCAAAATTGGTTTGGCC
TGTTTGCTACGAAGCTTGGGAGTTGTACTTCCCACACCTTTGCTACTGAGACACTATCACTACTGCC
ATGGCAATGCCCTCTCTCACGCCTTCTGTTTGACCAAGGATGTTCTAAGATTATCCTGTACAGATGC
CAGGACCAACAGTATTTATGGGCTTTGTGTAGTCATTGCCACACTAGGTGTGGATTCAATCTTCATA
CTTCTTTCTTATGTTCTGATTCTTAATACTGTGCTGGATATTGCATCTCGTGAAGAGCAGCTAAAGG
CACTCAACACATGTGTATCCCATATCTGTGTGGTGCTTATCTTCTTTGTGCCAGTTATTGGGGTGTC
AATGGTCCATCGCTTTGGGAAGCATCTGTCTCCCATAGTCCACATCCTCATGGCAGACATGTACCTT
CTTCTTCCCCCAGTCCTTAACCTATTGTCTATAGTGTGAGAACAAGCAGATTTCGTCTAGGAATTC
TCCACAAGTTTGCTCTAAGGAGGAGGTTTAAAGTAACCTCTGTCTCCAACCTTTTC

The SignalP, Psort and/or Hydropathy profile for the disclosed GPCR4b Olfactory Receptor-like protein predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The SignalP shows a signal sequence is coded for in the first 36 amino acids with a cleavage site at between amino acids 40 and 41, between LVA/FM in Table 4G. The molecular weight of GPCR4b is 35386.7 Da. This is typical of this type of membrane protein.

Table 4G. GPCR4b Amino Acid Sequence (SEQ ID NO:13)

MGDWNNSDAVEPIFILRGFPGLEYVHSWLSILFCLAYLVAFMGNVTILSVIWISSLHQPYYFISILAVNDLG
MSLTSLPTMLAVLWLDAPETIASACYAQLFFIHTFTFLESSVLLAMAFDRFVAICHPLHYPTILNSVIGKIGL
ACLLRSLGVVLPPLLLRHYHYCHGNALSHAFCLHQDVLRLSCTDARTNSIYGLCVVIATLGVDISIFILLSYVL
ILNTVLDIASREEQLKALNTCVSHICVVLIFFPVIGVSMVHRFGKHLSPIVHILMADMYLLLPVLPVIVYSV
RTKQIRLGLHFKFVLRFRF

BLASTP alignments also showed high homology between GPCR4b and other proteins as shown in Table 4H.

Table 4H. BLASTP results for GPCR4b

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
SPTREMBL- ACC:Q9YH55	OLFACTORY RECEPTOR- LIKE PROTEIN COR3'BETA - Gallus gallus (Chicken),	319	159/301 (52%)	217/301 (72%)	2.7e-85
SPTREMBL- ACC:Q9WVN4	MOR 5'BETA1 - Mus musculus (Mouse)	311	160/303 (52%)	219/303 (72%)	2.2e-83
ACC:Q9Y5P1	HOR 5'BETA3 - Homo sapiens (Human)	312	144/312 (46%)	221/312 (70%)	3.4e-78

The disclosed GPCR4b protein has good identity with a number of olfactory receptor proteins, as shown in Table 4I.

Table 4I. BLASTX results for GPCR4b

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P (N)	N
ptnr:SP TREMBL-ACC:Q9YH55 OLFACTORY RECEPTOR-LIKE PROTE.	+2	854	1.6e-84	1
ptnr:SP TREMBL-ACC:Q9WVN4 MOR 5'BETA1 - Mus musculus (M)	+2	836	1.3e-82	1
ptnr:SP TREMBL-ACC:O88628 PUTATIVE G-PROTEIN COUPLED RE.	+2	831	4.5e-82	1
ptnr:SP TREMBL-ACC:Q9WVN5 MOR 5'BETA2 - Mus musculus (M)	+2	828	9.3e-82	1
ptnr:SP TREMBL-ACC:Q9WVN6 MOR 5'BETA3 - Mus musculus (M)	+2	823	3.1e-81	1
ptnr:SP TREMBL-ACC:Q9Y5P1 HOR 5'BETA3 - Homo sapiens (H)	+2	787	2.1e-77	1
ptnr:SP TREMBL-ACC:Q9WU90 ODORANT RECEPTOR S19 - Mus mu.	+2	760	1.5e-74	1
ptnr:SP TREMBL-ACC:Q9WVD9 MOR 3'BETA1 - Mus musculus (M)	+2	751	1.3e-73	1
ptnr:SP TREMBL-ACC:Q9WU89 ODORANT RECEPTOR S18 - Mus mu.	+2	749	2.2e-73	1
ptnr:SP TREMBL-ACC:Q9Y5P0 HOR 5'BETA1 - Homo sapiens (H)	+2	745	5.8e-73	1
ptnr:SP TREMBL-ACC:Q9WVD7 MOR 3'BETA3 - Mus musculus (M)	+2	710	3.0e-69	1
ptnr:SP TREMBL-ACC:Q9WVD8 MOR 3'BETA2 - Mus musculus (M)	+2	705	1.0e-68	1
ptnr:SP TREMBL-ACC:Q9WU93 ODORANT RECEPTOR S46 - Mus mu.	+2	703	1.6e-68	1
ptnr:TREMBLNEW-ACC:CAB89291 OLFACTORY RECEPTOR - Homo	+2	532	1.8e-54	2

GPCR4c

The disclosed GPCR4c (also referred to herein as AC020597B2) is encoded by a nucleic acid, 994 nucleotides long (SEQ ID NO:16). An open reading frame was identified beginning with an ATG initiation codon at nucleotides 23-25 and ending with a TAA codon at nucleotides 968-970. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4J, and the start and stop codons are in bold letters. The encoded protein having 312 amino acid residues is presented using the one-letter code in Table 4B (SEQ ID NO:12).

Table 4J. GPCR4c Nucleotide Sequence (SEQ ID NO:14).

TGCTGAATTACTCAAAGTCACTATGGGAGACTGGAATAACAGTGATGCTGTGGAGCCCATATTATCCTGAGGGG
TTTTCTGAGTGGAGTATGTTTCATTCTTGGCTCTCCATCCTCTTCTGTCTTGCATATTTGGTAGCATTTATGGG
TAATGTTACCATCCTGTCTGTTCATTTGGATAGAACCTCTCTCCATCAGCCCATGTATTACTTTATTTCCATCTT
GGCAGTGAATGACCTGGGGATGTCCCTGTCTACACTTCCACCATGCTTGTCTGTGTATGGTTGGATGCTCCAGA
GATCCAGGCAAGTGCTTGCTATGCTCAGCTGTTCTTCATCCACACATTACATTCCCTGGAGTCTCAGTGTGCT
GGCCATGGCCTTTGACCGTTTTGTTGCTATCTGCCATCCACTGCACTACCCACCATCCTCACCACAGTGTAAT
TGGCAAAATTGGTTTGGCCTGTTTGCTACGAAGCTTGGGAGTTGTACTTCCACACCTTTGCTACTGAGACACTA
TCCTACTGCTCCATGGCAATGCCCTCTCTCAGCCTTCTGTTTGCACCAGGATGTTCTAAGATTATCCTGTACAGA
TGCCAGGACCAACAGTATTTATGGGCTTTGTGTAGTCATTGCCACACTAGGTGTGGATTCAATCTTCATACTTCT
TTCTTATGTTCTGATTCTTAATACTGTGCTGGATATTGCATCTCGTGAAGAGCAGCTAAAGGCACTCAACACATG
TGTATCCCATATCTGTGTGGTGCTTATCTTCTTGTGCCAGTTATGGGGTGTCAATGGTCCATCGCTTTGGGAA
GCATCTGTCTCCCATAGTCCACATCCTCATGGCAGACATCTACCTTCTTCTTCCCCAGTCCCTTAACCCATTGT
CTATAGTGTGAGAACAAAGCAGATTTCGTCTAGGAATTCACACAAGTTTGTCTTAAGGAGGAGGTTTTAAGTAAC
CTCTGTCTCCAACTTTTC

The encoded protein is the same as for GPCR4a and is disclosed above in Table 4B.

Unless specifically addressed as GPCR4a or GPCR4b, any reference to GPCR4 is assumed to encompass all variants. Residue differences between any GPCRX variant sequences herein are written to show the residue in the “a” variant and the residue position

with respect to the “a” variant. In all following sequence alignments, the GPCR4a protein sequence was used.

The disclosed GPCR4 protein (SEQ ID NO:12) also has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 4J.

Table 4J. BLAST results for GPCR4						
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect	
gi 11908220 gb AAG41684.1 (AF133300)	MOR 3'Beta4 [Mus musculus]	303	174/307 (56%)	223/307 (71%)	2e-81	
gi 11908225 gb AAG41688.1 (AF071080)	Mor 5'Beta5 [Mus musculus]	317	160/294 (54%)	215/294 (72%)	3e-79	
gi 11908214 gb AAG41679.1 (AF137396)	HOR5'Beta11 [Homo sapiens]	314	152/294 (51%)	226/294 (76%)	9e-79	
gi 11908213 gb AAG41678.1 (AF137396)	HOR5'Beta12 [Homo sapiens]	312	160/296 (54%)	211/296 (71%)	2e-78	
gi 11908218 gb AAG41683.1 (AF137396)	HOR5'Beta5 [Homo sapiens]	312	160/300 (53%)	220/300 (73%)	2e-77	

This information is presented graphically in the multiple sequence alignment given in Table 4K (with GPCR4 being shown on line 1) as a ClustalW analysis comparing GPCR4 with related OR sequences.

Table 4K Information for the ClustalW proteins:

- 1) GPCR4A (SEQ ID NO:12)
- 2) GPCR4B (SEQ ID NO:13)
- 3) >gi|11908220|gb|AAG41684.1| (AF133300) MOR 3'Beta4 [Mus musculus]
- 4) >gi|11908225|gb|AAG41688.1| (AF071080) Mor 5'Beta5 [Mus musculus]
- 5) >gi|11908214|gb|AAG41679.1| (AF137396) HOR5'Beta11 [Homo sapiens]
- 6) >gi|11908213|gb|AAG41678.1| (AF137396) HOR5'Beta12 [Homo sapiens]
- 7) >gi|11908218|gb|AAG41683.1| (AF137396) HOR5'Beta5 [Homo sapiens]

10	20	30	40	50	60	70	80	90	100	110	120
GPCR4a	--MGDWNNSDAVEPIFHLRGFPGLSYVHSMESLFCAYLVAFMGNVHLSVIWIESSLE										
GPCR4b	--MGDWNNSDAVEPIFHLRGFPGLSYVHSMESLFCAYLVAFMGNVHLSVIWIESSLE										
gi 11908220 gb AAG41684.1	--MATSNSSTIVSSTFYLTCIPCYEEFHWISIPFCFLYLVGTTGNCMLIHVVRTDPRLE										
gi 11908225 gb AAG41688.1	MKVSIPPRANFSYAIFELTGFPGLEWAEHWSLPIFGYFVAHMCNAILHLVVRTDPSLE										
gi 11908214 gb AAG41679.1	--MLGLNGTPFPATLQLTCTDCTGTGLTWALIFCLLYVTSVGNLSIITVFWEPALH										
gi 11908213 gb AAG41678.1	---MGLFNVTHPAFLTLTGIPGLESSSEHWSLGPLCMYAVALGENTVLLQAVRVPSLE										
gi 11908218 gb AAG41683.1	-----MSSSSSHPFELTGFPGLEEAHWSLFFFLFMYLSILFNGTHLLLIKEDHNLH										
GPCR4a	CPMYFFESLAVNDLGSLSSTLPTMLAVLWLDAPETQASACYACFFFIHFFFLSSSVLL										
GPCR4b	CPMYFFESLAVNDLGSLSSTLPTMLAVLWLDAPETQASACYACFFFIHFFFLSSSVLL										
gi 11908220 gb AAG41684.1	CPMYFFLAMLSTLDMASLPTMMSLFRVLWSISRELQFNIQVQVFLHFFSFTSSVLL										
gi 11908225 gb AAG41688.1	CPMYFFLAMLSTLDMASLPTMMSLFRVLWSISRELQFNIQVQVFLHFFSFTSSVLL										
gi 11908214 gb AAG41679.1	CPMYFFLAMLSTLDMASLPTMMSLFRVLWSISRELQFNIQVQVFLHFFSFTSSVLL										
gi 11908213 gb AAG41678.1	CPMYFFLAMLSTLDMASLPTMMSLFRVLWSISRELQFNIQVQVFLHFFSFTSSVLL										
gi 11908218 gb AAG41683.1	CPMYFFLAMLSTLDMASLPTMMSLFRVLWSISRELQFNIQVQVFLHFFSFTSSVLL										

and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon.

The nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette and/or other pathologies and disorders of the like. The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the OR -like protein may be useful in gene therapy, and the OR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome. Other GPCR-4 diseases and disorders are contemplated.

The novel nucleic acid encoding OR-like protein, and the OR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods and other diseases, disorders and conditions of the like. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

GPCR5

GPCR5 includes a family of three similar nucleic acids and three similar proteins disclosed below. The disclosed nucleic acids encode GPCR, OR-like proteins.

GPCR5a

The disclosed novel GPCR5a nucleic acid of 985 nucleotides (also referred to as 1 AC020597_C) is shown in Table 5A. An ORF begins with an ATG initiation codon at nucleotides 27-29 and ends with a TAA codon at nucleotides 960-962. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 5A, and the start and stop codons are in bold letters.

Table 5A. GPCR5a Nucleotide Sequence (SEQ ID NO:15)
GTTCTCCTACACTGTGATTGGAAAAATGTTTTATCACAACAAGAGCATATTTACCCAGTCACATTTTCTCTCA TTGGAATCCCAGGTCTGGAAGACTTCCACATGTGGATCTCCGGGCCTTTCTGCTCTGTTACCTTGTGGCTTTGC TGGGCAATGCCACCATTCTGCTAGTCATCAAGGTAGAACAGACTCTCCGGGAGCCCATGTTCTACTTCTGGCCA TTCTTTCCACTATTGATTTGGCCCTTTCTGCAACCTCTGTGCCTCGCATGCTGGGTATCTTCTGGTTTGATGCTC ACGAGATTAACTATGGAGCTTGTGTGGCCAGATGTTTCTGATCCATGCCTTCACTGGCATGGAGGCTGAGGTCT TACTGGCTATGGCTTTTGACCGTTATGTGGCCATCTGTGCTCCACTACATTACGCAACCATCTTGACATCCCTAG TGTTGGTGGGCATTAGCATGTGCATTGTAATTCGTCCCGTTTACTTTACACTTCCCATGGTCTATCTTATCTACC GCCTACCCTTTGTGAGGCTCACATAATAGCCATTCTACTGTGAGCACATGGGCATTGCAAAATTGTCCTGTG GAAACATTCGTATCAATGGTATCTATGGGCTTTTGTAGTTTCTTTCTTTGTTCTGAACCTGGTGCTCATTGGCA TCTCGTATGTTTACATTCTCCGTGCTGCTTCCGCCTCCCATCACATGATGCTCAGCTAAAAGCCCTAAGCACGT GTGGCGCTCATGTTGGAGTCATCTGTGTTTTCTATATCCCTTCAGTCTTCTCTTTCCTTACTCATCGATTGGAC ACCAAATACAGGTTACATTACATTCTGTTGCCAATCTCTATTGATTATCCACCCTCTCTCAACCCCATCA TTTATGGGGTGAGGACCAACAGATTGAGAGCGAGTGCTCTATGTTTTTACTAAAAAATAAGACTCTTACCATG TTATTTTACT

The GPCR5a protein encoded by SEQ ID NO:15 has 311 amino acid residues and is presented using the one-letter code in Table 5B. The Psort profile for GPCR5a predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a

certainty of 0.6400. The most likely cleavage site for a peptide is between amino acids 49 and 50: between ILL-VI based on the SignalP result.

Table 5B. Encoded GPCR5a protein sequence (SEQ ID NO:16)

MFYHNKSI FHPVTFFLIGIPGLEDFHMWISGPFCSVYL VALLGNATILLVIKVEQTLREPMFYFLAILSTIDL ALSATSVPRMLGIFWFDAHEINYGACVAQMFLIHAFTGMEAEVLLAMAFDRYVAICAPLHYATILTSVLVGI SMCIVIRPVLLTLPVYLIYRLPFCQAHIIAHSYCEHMGIAKLSCGNIRINGIYGLFVVSFFVLNLVLIGISY VYILRAVFRLLPSHDAQLKALSTCGAHVGVICVFYIPSVFSFLTHRFGHQIPGYIHILVANLYLIIPPSLNPII YGVRTKQIRERVLYVFTKK
--

5 The disclosed nucleic acid sequence for GPCR5 has 633 of 989 bases (64%) identical to a *Mus musculus* GPCR mRNA (GENBANK-ID: AF121979) ($E = 7.2e^{-60}$).

10 The full GPCR5a amino acid sequence has 174 of 303 amino acid residues (57%) identical to, and 231 of 303 residues (76%) positive with, the 318 amino acid residue odorant receptor S46 protein from *Mus musculus* (ptnr:SPTREMBL-ACC: Q9WU93) ($E = 1.9e^{-92}$) and 146 of 310 amino acid residues (47%) identical to, and 212 of 310 residues (68%) positive with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (ptnr: TREMBLNEW-ACC:AAD51279) ($E = 2.1e^{-77}$)

GPCR5b

15 GPCR5a (CG53668-02) was subjected to an exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide GPCR5b, which is also referred to as CG53668-02.

25 The nucleotide sequence for GPCR5b (947 bp, SEQ ID NO:17) is presented in Table 5C.

Table 5C. GPCR5b Nucleotide Sequence (SEQ ID NO:17)

TGAAAAATGTTTTATCACAACAAGAGCATATTTACCCAGTCACATTTTTCTCATTGGAATCCCA
GGTCTGGAAGACTTCCACATGTGGATCTCCGGGCTTTCTGCTCTGTTTACCTTGTGGCTTTGCTG
GGCAATGCCACCATTCTGCTAGTCATCAAGGTAGAACAGACTCTCCGGGAGCCCATGTTCTACTTC
CTGGCCATTCTTTCCACTATTGATTTGGCCCTTTCTACAACCTCTGTGCCTCGCATGCTGGGTATC
TTCTGGTTTGATGCTCACGAGATTAAGTATGGAGCTTGTGTGGCCAGATGTTTCTGATCCATGCC
TTCATGGCATGGAGGCTGAGGTCTTACTGGCTATGGCTTTTGACCGTTATGTGGCCGTCTGTGC
TCCACTACATTACGCAACCATCTTGACATCCCAAGTGTGGTGGGCATTAGCATGTGCATTGTAAT
CCGTCCCGTTTTACTTACACTTCCCATGGTCTATCTTATCTACCGCTACCTTTTGTGAGGCTCAC
ATAATAGCCCATCTACTGTGAGCACATGGGCATTGCAAAATTGTCCTGTGGAAACATTGTCATC
AATGGTATCTATGGGCTTTTGTAGTTTCTTCTTTGTTCTGAACCTGGTGCTCATTGGCATCTCG
TATGTTTACATTCTCCGTGCTGTCTTCCGCCTCCCATCACATGATGCTCAGCTAAAAGCCCTAAGCA
CGTGTGGCGCTCATGTTGGAGTCATCTGTGTTTTCTATATCCCTTCACTTCTCTTTTCTTACTCA
TCGATTTGGACACCAAATACCAGTTACATTACATTCTTGTGCAATCTCTATTTGATTATCCCA
CCCTCTCTCAACCCATCATTTATGGGGTGAGGACCAACAGATTGAGAACGAGTGCTCTATGTT
TTTACTAAAAATAGACTA

The encoded GPCR5b protein is presented in Table 5D. The disclosed protein is 311 amino acids long and is denoted by SEQ ID NO:18. GPCR5b differs from GPCR5a by 3 amino acid changes in positions 77 A->T, 128 I->V and 141 L->Q. Like GPCR5a, the Psort profile for GPCR5b predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The most likely cleavage site for a peptide is between amino acids 49 and 50, *i.e.*, at the slash in the amino acid sequence ILL-VI based on the SignalP result.

Table 5D. Encoded GPCR5b protein sequence (SEQ ID NO:18)

MFYHNKSI FHPVTF FLIGIPGLEDFH MWISGPFC SVYLVALLGNATILLVIKVEQTLREPMFYFLAILSTIDLAL
STTSVPRMLGIFWFD AHEINYGACVAQMFLIHAFTGMEAEVLLAMAFDRYVAVCAPLHYATILTSQVLVGISMCI
VIRPVLLTLP MVYLIYRLPFCQAHIIAHSYCEHMGIAKLSCGNIRINGIYGLFVVSFFVLNLVLIGISYVYILRA
VFRLPSHDAQLKALSTCGAHVGVICVFYIPSVFSFLTHFRGHQIPGYIHILVANLYLIIPPSLNPIIYGVRTKQI
RERVLYVFTKK

The disclosed nucleic acid sequence for GPCR5b has 598 of 921 (64%) identical to a *Mus musculus* odorant receptor S46 mRNA (GENBANK-ID: AF121979) ($E=9.5e^{-60}$).

The full GPCR5 amino acid sequence has 174 of 303 amino acid residues (57%) identical to, and 233 of 303 (76%) positive with, the 318 amino acid residue odorant receptor S46 protein from *Mus musculus* (ptnr:SPTREMBL-ACC: Q9WU93) ($E=9.7e^{-92}$).

The disclosed GPCR5 protein also has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 5E.

GPCR5c

Another nucleotide sequence resulted when GPCR5a (AC020597_C) was subjected to an exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide the sequence reported below, which is designated as Accession Number AC020597B_da1, or GPCR5c.

The nucleotide sequence for GPCR5c (945 bp, SEQ ID NO:19) is presented in Table 5E.

Table 5E. GPCR5c Nucleotide Sequence (SEQ ID NO:19)

GAAAAATGTTTATCACAACAAGAGCATATTTACCCAGTCACATTTTCTCATTGGAATCCCAGGTCTG GAAGACTTCCACATGTGGATCTCCGGGCTTTCTGCTCTGTTTACCTTGCGGCTTGTCTGGGCAATGCCAC CATTCTGCTAGTCATCAAGGTAGAACAGACTCTCCGGGAGCCCATGTTCTACTTCCTGGCCATTCTTTCCA CTATTGATTTGGCCCTTTCTACAACCTCTGTGCCTCGCATGCTGGGTATCTTCTGGTTTGATGCTCACGAG ATTAACATATGGAGCTTGTGTGGCCAGATGTTTCTGATCCATGCCTTCACTGGCATGGAGGCTGAGGTCTT ACTGGCTATGGCTTTTGACCGTTATGTGGCCGTCTGTGCTCCACTACATTACGCAACCATCTTGACATCCC AAGTGTGGTGGGCATTAGCATGTGCATTGTAATTCGTCCCGTTTACTTACACTTCCCATGGTCTATCTT ATCTACCGCCTACCCCTTTGTCAAGGCTCACATAATAGCCCATTCCTACTGTGAGCACATGGGCATTGCAAA ATTGTCCTGTGGAAACATTTCGTATCAATGGTATCTATGGGCTTTTGTAGTTTCTTCTTGTGTTCTGAACC TGGTGTCTCATTGGCATCTCGTATGTTTACATTCTCCGTGCTGTCTTCCGCCTCCCATCATATGATGCTCAG CTAAAAGCCCTAAGCACGTGTGGCGCTCATGTTGGAGTCATCTGTGTTTCTATATCCCTTCAGTCTTCTC TTTCTTACTCATCGATTGGACACCAAATACCAGGTTACATTACATTCTTGTGCCAATCTCTATTGTA TTATCCCAACCTCTCTCAACCCCATCATTTATGGGGTGAGGACCAACAGATTCGAGAACGAGTGCTCTAT GTTTTTACTAAAAATAAGACT

The coding region of GPCR5c is from nucleotide 6 to 938, giving the encoded GPCR5c protein, as presented in Table 5F. The disclosed protein is 311 amino acids long and is denoted by SEQ ID NO: 20. The Psort profile for GPCR5c predicts that this sequence has a signal peptide and is likely to be localized at the endoplasmic reticulum with a certainty of 0.6850. The most likely cleavage site for a peptide is between amino acids 49 and 50, ILL-VI based on the SignalP result.

Table 5F. Encoded GPCR5c protein sequence (SEQ ID NO:20)

MFYHNKSI FHPVTFFLIGIPGLEDFHMWISGPFCSVYLAALLGNATILLVIKVEQTLREPMFYFLAILSTI
DLALSTTSVPRMLGIFWFDAHEINYGACVAQMFLIHAFTHGMEAEVLLAMAFDRYVAVCAPLHYATILTSQV
LVGISMCI VIRPVLLTLPVMVLIYRLPFCQAHIIAHSYCEHMGIAKLSCGNIRINGIYGLFVVVSFFVLNLV
LIGISYVYILRAVFRPLPSHDAQLKALSTCGAHVGVICVFYIPSVFSFLTRFHGHQIPGYIHILVANLYLII
PPSLNPIIYGVRTKQIRERVLYVFTKK

The full GPCR5c amino acid sequence has 174 of 303 amino acid residues (57%) identical to, and 232 of 303 residues (76%) positive with, the 318 amino acid residue odorant receptor S46 protein from *Mus musculus* (ptnr:SPTREMBL-ACC: Q9WU93) ($E=2.2e^{-92}$) and 146 of 310 amino acid residues (47%) identical to, and 212 of 310 residues (68%) positive with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (ptnr: SPTREMBL-ACC:Q9UKL2) ($E=2.1e^{-77}$).

Possible SNPs found for GPCR5c are listed in Table 5G.

Table 5G: SNPs

Base Position	Base Before	Base After
63	T	C(3)
94	C	T(4)
110	C	T(4)
114	A	G(4)
157	G	T(2)
170	T	C(2)
197	G	C(2)
242	T	C(3)
262	G	A(2)
290	G	A(3)
299	G	C(3)
314	G	T(2)
316	A	C(2)
329	C	T(2)
332	A	C(3)
333	T	A(3)
356	A	C(3)
376	A	G(3)
377	C	T(2)
396	A	G(3)
428	C	T(2)
453	A	G(2)

The disclosed GPCR5 protein (SEQ ID NO:19) has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented

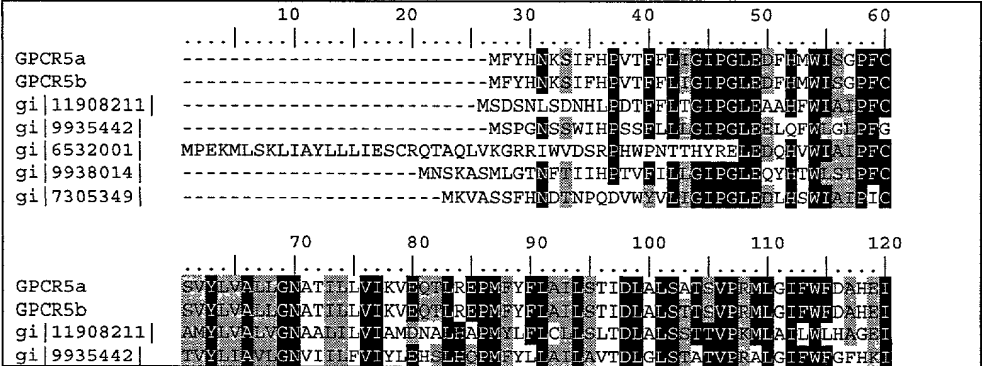
in Table 5I. Unless specifically addressed as GPCR5a GPCR5b, or GPCR5c, any reference to GPCR5 is assumed to encompass all variants. All GPCR5 proteins have significant homology to olfactory receptor (OR) proteins: The homology information from BLASTX alignments for the proteins in the ClustalW is presented in Table 5H.

Table 5H. BLAST results for GPCR5					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 11908211 gb AAG41676.1 (AF137396)	HOR 5'Beta14 [Homo sapiens]	318	166/297 (55%)	217/297 (72%)	7e-81
gi 9935442 ref NP_064688.1	odorant receptor S46 gene [Mus musculus]	318	168/303 (55%)	218/303 (71%)	1e-80
gi 6532001 gb AAD27596.2 AF121976_1 (AF121976)	odorant receptor S19 [Mus musculus]	339	162/291 (55%)	208/291 (70%)	1e-79
gi 9938014 ref NP_064686.1	odorant receptor S18 gene [Mus musculus]	321	158/299 (52%)	218/299 (72%)	1e-79
gi 7305349 ref NP_038647.1	olfactory receptor 67 [Mus musculus]	326	164/309 (53%)	222/309 (71%)	5e-78

This information is presented graphically in the multiple sequence alignment given in Table 5K (with GPCR5a being shown on line 1 and GPCR5b on line 2) as a ClustalW analysis comparing GPCR5 with related protein sequences.

Table 5I Information for the ClustalW proteins:

- 1) GPCR5a (SEQ ID NO:19)
- 2) GPCR5b
- 3) >gi|11908211|gb|AAG41676.1| (AF137396) HOR 5'Beta14 [Homo sapiens]
- 4) >gi|9935442|ref|NP_064688.1| odorant receptor S46 gene [Mus musculus]
- 5) >gi|6532001|gb|AAD27596.2|AF121976_1 (AF121976) odorant receptor S19 [Mus musculus]
- 6) >gi|9938014|ref|NP_064686.1| odorant receptor S18 gene [Mus musculus]
- 7) >gi|7305349|ref|NP_038647.1| olfactory receptor 67 [Mus musculus]



Consensus 7tm 1 domain S L P P L F S ----- L L T V E E G N T ----- T V C L I D F P E E S V K -----

5 GPCR5
Consensus 7tm 1 domain

190 200 210 220 230 240

INGIYGHFVWSFFVFNNAVLTGTSVYVTLRAVFR-----
-RSYVLLSTVGGELPPEVYVLCVTRILLETTRKSARSQRS-----

10 GPCR5
Consensus 7tm 1 domain

250 260 270 280 290 300

-----LPSHDNQLKALSTCGAHVGVICVFYVPS
-----LKRRSSSERKAAKMLLVVVVVFVLCWLEY

15 GPCR5
Consensus 7tm 1 domain

310 320 330 340 350

-----VFSFLLTHRFGHQ-----IPGYIHTLNAVLYLTTPPSLNPIIY
-----HIVLLDSLCLLSIWR-----VLPTALLETWLWAYNSCLNPIIY

The nucleic acids and proteins of GPCR5 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the GPCR (or olfactory-receptor) like protein may be useful in gene therapy, and the receptor-like protein may be useful when administered to a subject in need thereof. The nucleic acids and proteins of the invention are also useful in potential therapeutic applications used in the treatment of developmental diseases, MHCII and III diseases (immune diseases), taste and scent detectability disorders, Burkitt's lymphoma, corticoneurogenic disease, signal transduction pathway disorders, retinal diseases including those involving photoreception, cell growth rate disorders, cell shape disorders, feeding disorders, potential obesity due to over-eating, potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, allergies, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease, multiple sclerosis, Albright hereditary osteodystrophy, angina pectoris, myocardial infarction, ulcers, benign prostatic hypertrophy, psychotic and neurological disorders (including anxiety, schizophrenia, manic depression, delirium, dementia, and severe mental retardation), dentatorubro-pallidoluysian atrophy (DRPLA), hypophosphatemic rickets, autosomal dominant (2) acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. Other GPCR-related diseases and disorders are contemplated.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in

need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from developmental diseases, MHCII and III diseases (immune diseases), taste and scent detectability disorders, Burkitt's lymphoma, corticoneurogenic disease, signal transduction pathway disorders, retinal diseases including those involving photoreception, cell growth rate disorders, cell shape disorders, feeding disorders, potential obesity due to over-eating, potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, allergies, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease, multiple sclerosis, Albright hereditary osteodystrophy, angina pectoris, myocardial infarction, ulcers, benign prostatic hypertrophy, psychotic and neurological disorders (including anxiety, schizophrenia, manic depression, delirium, dementia, and severe mental retardation), dentatorubro-pallidoluyian atrophy (DRPLA), hypophosphatemic rickets, autosomal dominant (2) acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. For example the disclosed GPCR5 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR5 epitope is from about amino acids 170 to 200. In another embodiment, a GPCR5 epitope is from about amino acids 230 to 250. In additional embodiments, GPCR5 epitopes are from amino acids 270 to 310. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

GPCR6

GPCR6a

The disclosed novel GPCR6a nucleic acid of 1012 nucleotides (also referred to as AC020597_D) is shown in Table 6A. An open reading begins with an ATG initiation codon

at nucleotides 16-18 and ends with a TGA codon at nucleotides 991-993. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 6A, and the start and stop codons are in bold letters.

Table 6A. GPCR6a Nucleotide Sequence (SEQ ID NO:21)

GCATTCAACAAGCAGGATGTTCTTCCCAATGACACCCAGTTTCACCCCTCCTCCTTCTGTTGCTGGGGATCCCA
GGACTAGAAACACTTCACATCTGGATCGGCTTCCCTTCTGTGCTGTGTACATGATCGCACTCATAGGGAACCTC
ACTATTCTACTTGTGATCAAGACTGACAGCAGCCTACACAGCCCATGTTCTACTTCTGGCCATGTTGGCCACC
ACTGATGTGGGTCTCTCAACAGCTACCATCCCTAAGATGCTTGAATCTTCTGGATCAACCTCAGAGGGATCATC
TTTGAAGCCTGCCTCACCAGATGTTTTTTATCCACAACCTCACACTTATGGAGTCAGCAGTCCTTGTGGCAATG
GCTTATGACAGCTATGTGGCCATCTGCAATCCACTCCAATATAGCGCCATCCTCACCAACAAGGTTGTTTCTGTG
ATTGGTCTTGGTGTGTTTGTGAGGGCTTTAATTTTCGTCATTCCTCTATACTTCTTATATTGCGGTTGCCCTTC
TGTGGGAATCATGTAATCCCCACACCTACTGTGAGCACATGGGTCTTGCTCATCTATCTTGTGCCAGCATCAAA
ATCAATATTATTTATGGTTTATGTGCCATTGTAATCTGGTGTGTTGACATCACAGTCATTGCCCTCTCTTATGTG
CATATTCTTGTGCTGTTTTCCGTCCTTACTCATGAGCCCCGACTCAAGTCCCTCAGCACATGTGGTTCACAT
GTGTGTGTAATCCTTGCCTTCTATACACAGCCCTCTTTCTTTATGACTCATTGCTTTGGCCGAAATGTGCCC
CGCTATATCCATATACTCCTAGCCAATCTCTATGTTGTGGTGGCCCAATGCTCAATCCTGTCATATATGGAGTC
AGAACCAAGCAGATCTATAAATGTGTAAAGAAAATATTATGCAGGAACAAGGAATGAAAAGGAAGAGTACCTA
ATACATACGAGGTTCTGAATGCAATTTATGAAATTT

The disclosed nucleic acid sequence has 662 of 1005 bases (65%) identical to a *Mus musculus* odorant receptor S46 mRNA (GENBANK-ID: AF121979) (E value = $1.8e^{-70}$).

The GPCR6a protein encoded by SEQ ID NO:21 has 325 amino acid residues, and is presented using the one-letter code in Table 6B (SEQ ID NO:22).

Table 6B. Encoded GPCR6a protein sequence (SEQ ID NO:22).

MFLPNDTQFHPSSFLLGLGIPGLETLHIWIGFPFCAYMIALIGNFTILLVIKTDSSLHQPMPFYFLAMLATTDVGLS
TATIPKMLGIFWINLRGIIFEACLTQMFFIHNFTLMESAVLVAMAYDSYVAICNPLQYSAILTNKVVSVIGLGVFV
RALIFVIPSILLILRLPFCGNHVIPTTYCEHMGHLHLSCASIKINIYGLCAICNLVFDITVIALSYVHILCAVFR
LPTHEPRLKSLSTCGSHVCVILAFYTPALFSFMTHCFGRNVPRIYHILLANLYVVVPMLNPVIYGVRTK
QIYKCVKKILLQEQGMEKEEYLIHTRF

The full amino acid sequence of GPCR6a was found to have 192 of 309 amino acid residues (62%) identical to, and 239 of 309 residues (77%) positive with, the 318 amino acid residue Odorant Receptor S46 from *Mus musculus* (SPTREMBL-ACC:Q9WU93) (E value = $2.3e^{-103}$), and 152 of 302 amino acid residues (50%) identical to, and 211 of 302 residues (69%) positive with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (TREMBLNEW-ACC:AAD51279) (E value = $1.8e^{-80}$)

GPCR6b

In the present invention, the target sequence identified previously, Accession Number AC020597D, was subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward

primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide the sequence reported below, which is designated Accession Number AC020597D1. The resulting nucleotide sequence differs in 5 of 1012 bases given by Accession Number AC020597D, which leads to a difference in the amino acid sequence at residues 234 and 264.

The disclosed novel GPCR6b nucleic acid of 1012 nucleotides (also referred to as AC020597_D1) is shown in Table 6C. An open reading begins with an ATG initiation codon at nucleotides 16-18 and ends with a TGA codon at nucleotides 991-993. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 6C, and the start and stop codons are in bold letters.

Table 6C. GPCR6b Nucleotide Sequence (SEQ ID NO:23)

GCATTCACAAGCAGGATGTTCCCTTCCCAATGACACCCAGTTTCACCCCTCCTCCTTCTGTTGCTGGGGATCCCA
GGACTAGAAACACTTCACATCTGGATCGGCTTTCCCTTCTGTGCTGTGTACATGATCGCACTCATAGGGAACCTC
ACTATTCTACTTGTGATCAAGACTGACAGCAGCCTACACCAGCCCATGTTCTACTTCCTGGCCATGTTGGCCACC
ACTGATGTGGGTCTCTCAACAGCTACCATCCCTAAGATGCTTGAATCTTCTGGATCAACCTCAGAGGGATCATC
TTTGAAGCCTGCCTCACCAGATGTTTTTATCCACAACCTCACACTTATGGAGTCAGCAGTCCTTGTGGCAATG
GCTTATGACAGCTATGTGGCCATCTGCAATCCACTCCAATATAGCGCCATCCTCACCACAAGGTTGTTTCTGTG
ATTGGTCTTGGTGTGTTGTGAGGGCTTTAATTTTCGTCAATCCCTCTATACTTCTTATATTGCCGTTGCCCTTC
TGTGGGAATCATGTAATCCCCACACCTACTGTGAGCACATGGGTCTTGCTCATCTATCTTGTGCCAGCATCAAA
ATCAATATTATTTATGGTTTATGTGCCATTTGTAATCTAGTGTGACATCACAGTCATTGCCCTCTCTTATGTG
CATATTCTTTGTGCTGTTTTCCGTCTTCTACTCATGAAGCCGACTCAAGTCCCTCAGCACATGTGGTTACAT
GTGTGTGTAATCCTTGCCCTTCTATACACCAGCCCTCTTTTCTTTATGACTCATCGCTTTGGCCGAAATGTGCCC
CGCTATATCCATATACTCCTAGCCAATCTCTATGTTGTGGTGCCACCAATGCTCAATCCTGTATATATGGAGTC
AGAACCAAGCAGATCTATAAATGTGTGAAGAAAATATTATTGCAGGAACAAGGAATGGAAGGAAGAGTACCTA
ATACATACGAGTTCTGAATGCAATTTTATGAAATTT

The GPCR6b protein encoded by SEQ ID NO:21 has 325 amino acid residues, and is presented using the one-letter code in Table 6D (SEQ ID NO:24). The Psort profile for GPCR6b predicts that this sequence has a signal peptide and is likely to be localized at the endoplasmic reticulum with a certainty of 0.6850. It seems to have an uncleavable N-terminal signal sequence. The most likely cleavage site for a peptide, if there was one, is between amino acids 55 and 56, TDS-SL based on the SignalP result.

Table 6D. Encoded GPCR6b protein sequence (SEQ ID NO:24).

MFLPNDTQFHPSSFLLLGIPGLETLHIWIGFFPCAIVMIALIGNFTILLVIKTDSSLHQPMFYFLAMLATTDVGLS
TATIPKMLGIFWINLRGIIFEACLTQMFFIHNFTIMESAVLVAMAYDSYVAICNPLQYSAILTNKVVSIVIGLVFV

RALIFVIPSILLILRLPFCGNHVPHTYCEHMGHLAHLSCASIKINIYGLCAICNLVFDITVIALSYVHILCAVFR
 LPTHEARLKSLSCTCGSHVCVILAFYTPALFSFMTHRFGRNVPRYIHILLANLYVVVPPMLNPVIYGVRTKQIYKCV
 KKILLQEQGMEKEEYLIHTRF

BLASTP results include those listed in Table 6E.

Table 6E BLAST results for GPCR6					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
SPTREMBL-ACC:Q9WU93	ODORANT RECEPTOR S46 - Mus musculus	318	194/309 (62%)	241/309 (77%)	1.6e- 105
SPTREMBL-ACC:Q9WVD9	MOR 3'BETA1 - Mus musculus (Mouse)	326	172/298 (57%)	226/298 (75%)	4.4e- 94
SPTREMBL-ACC:Q9Y5P1	HOR 5'BETA3 - Homo sapiens (Human)	312	131/305 (42%)	195/305 (63%)	4.0e- 68

The disclosed GPCR6 protein has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 6E. The GPCR6 protein has significant identity to olfactory receptor (OR) proteins:

Table 6E. BLAST results for GPCR6					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 9935442 ref NP_064688.1	odorant receptor S46 gene [Mus musculus]	318	192/310 (61%)	239/310 (76%)	6e-98
gi 7305349 ref NP_038647.1	O olfactory receptor 67 [Mus musculus]	326	70/298 (57%)	224/298 (75%)	1e-86
gi 9938014 ref NP_064686.1	odorant receptor S18 gene [Mus musculus]	321	157/305 (51%)	225/305 (73%)	1e-83
gi 6532001 gb AAD27596.2 AF121976_1 (AF121976)	odorant receptor S19 [Mus musculus]	339	158/280 (56%)	209/280 (74%)	4e-83
gi 11908211 gb AAG41676.1 (AF137396)	HOR 5'Beta14 [Homo sapiens]	313	160/300 (53%)	221/300 (73%)	1e-82

This information is presented graphically in the multiple sequence alignment given in Table 6F (with GPCR6a being shown on line 1 and GPCR6b being shown on line 2) as a ClustalW analysis comparing GPCR6 with related protein sequences.

Table 6F Information for the ClustalW proteins:

- 1) GPCR6a (SEQ ID NO:25)
- 2) GPCR6b
- 3) gi|9935442|ref|NP_064688.1| odorant receptor S46 gene [Mus musculus]
- 4) gi|7305349|ref|NP_038647.1| olfactory receptor 67 [Mus musculus]
- 5) gi|9938014|ref|NP_064686.1| odorant receptor S18 gene [Mus musculus]

The presence of identifiable domains in GPCR6 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR6 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 6G with the statistics and domain description. The results indicate that GPCR6 has homology to the 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family) (as defined by Interpro) This indicates that the sequence of GPCR6 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

Table 6G. DOMAIN results for GPCR6

CD-Length = 254 residues, 100.0% aligned
Score = 77.4 bits (182), Expect = 1e-15

10	20	30	40	50	60	70	80	90	100	110	120
GPCR6	GNFTTL	LVIKTDSS	TH-QPMFY	FLAMLAT	TEVGL	STATIE	PKMIG	GIFWIN	-----	LRGII	
Consensus 7tm 1 domain	GNLLVL	LVTLRTKK	LR-TTNI	PLLNLA	VALFL	LLTLF	EWAL	YYLVGG	---	DWYF	SDA
15											
20	GPCR6	FEAC	TOMFF	HNFTL	MESAV	VMAYE	SYWAT	CNPL	VSAIL	LNKVV	SVGL
Consensus 7tm 1 domain	LCKLV	GALFVN	--GYA	SILLT	TAIST	RYEAT	VEPLR	YRRIR	EPAR	AKVIL	LVVPL
25	GPCR6	IFV	PSILLI	LRLP	FCGNH	-----	VIPHY	CEHMG	L---A	HLSC	ASIK
Consensus 7tm 1 domain	LLSE	PPPL	FSWLR	TVEEG	NT-----	TVCL	IDFP	-----	EE	SVK	---RSYVL
30	GPCR6	CNL	YFDIT	VIAL	SYVH	ILC-----					
Consensus 7tm 1 domain	VGF	ELPL	LVTL	LCYTR	ILR-----						
35	GPCR6										
Consensus 7tm 1 domain											
40	GPCR6	AVF	ELP	THEPR	-LKS	STCG	-----	SHV	CVIT	AFYT	ALFS
Consensus 7tm 1 domain	-TLR	SARS	QSRSL	KRRSS	SERKA	AKML	LVVV	VVF	LCLE	YHIV	LLDSL
45	GPCR6										
Consensus 7tm 1 domain											

The similarity information for the GPCR6 protein and nucleic acid disclosed herein suggest that GPCR6 may have important structural and/or physiological functions

characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon. The novel nucleic acid encoding GPCR6, and the GPCR6 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

The nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the OR -like protein may be useful in gene therapy, and the OR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary

retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The novel nucleic acid encoding OR-like protein, and the OR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR6 substances for use in therapeutic or diagnostic methods.

GPCR7

GPCR7a

A novel GPCR nucleic acid was identified by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs, and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR7 nucleic acid of 968 nucleotides (also referred to as AC025249_A) is shown in Table 7A. An open reading begins with an ATG initiation codon at nucleotides 6-8 and ends with a TAG codon at nucleotides 960-962. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 7A, and the start and stop codons are in bold letters.

Table 7A. GPCR7a Nucleotide Sequence (SEQ ID NO:25)

TCTTCATGATGGTGGATCCCAATGGCAATGAATCCAGTGCTACATACTTCATCCTAATAGGCCTCCCTGGTTAGAA
AGGCTCAGTTCTGGTTGGCCTTCCCATTTGTGCTCCCTCTACCTTATTGCTGTGCTAGGTAAGTGAACATCATCTACA
TTGTGCGGACTGAGCACAGCCTGCATGAGCCCATGTATATATTTCTTTGTCATGCTTTTCAGGCATGTGACATCCTCATCT
CCACCTCATCCATGCCCAAAATGCTGGCCATCTTCTGGTTCAATTCACACTACCATCCAGTTTGATGCTTGTCTGTAC
AGATGTTTGGCCATCCACTCCTTATCTGGCATGGAATCCACAGTGCTGCTGGCCATGGCTTTTGACCGCTATGTGGCCA
TCTGTCAACCACTGCGCCATGCCACAGTACTTACGTTGCCTCGTGTACCAAATTTGGTGGCTGCTGTGGTGCGGG
GGGCTGCACTGATGGCACCCCTTCTGTCTTCATCAAGCAGCTGCCCTTCTGCCGCTCCAATATCCTTTCCCATTCCT
ACTGCCTACACCAAGATGTGATGAAGCTGGCCTGTGATGATATCCGGGTCAATGTCGTCTATGGCCTTATCGTCATCA
TCTCCGCCATTGGCCTGGACTCACTTCTCATCTCCTTCTCATATCTGCTTATTCTTAAAGACTGTGTTGGGCTTGACAC
GTGAAGCCCAGGCCAAGGCATTTGGCACTTGCGTCTCTCATGTGTGTGCTGTGTTTATATTCTATGTACCTTTTCATTG
GATTGTCCATGGTGCATCGCTTTAGCAAGCGGCGTGACTCTCCGCTGCCCGTCATCTTGGCCAATATCTATCTGCTGG
TTCCTCCTGTGCTCAACCAATTGTCTATGGAGTGAAGACAAAGGAGATTCGACAGCGCATCCTTCGACTTTTCCATG
TGGCCACACACGCTTCAGAGCCCTAGGTGTCA

GPCR7a has high homology to several other nucleic acids including those in the BLASTN alignments described in Table 7B.

Table 7B. BLASTN results for GPCR7a				
Gene Index/ Identifier	Protein/ Organism	Length (bp)	Identity (%)	Expect
gb:GENBANK- ID:AF079864 acc:AF0 79864	Rattus norvegicus putative G- protein coupled receptor RA1c mRNA, complete cds	2910	596/879 (67%)	2.4e-68
gb:GENBANK- ID:AR009514 acc:AR0 09514	Sequence 1 from patent US 5756309	1474	594/874 (67%)	7.0e-69
patn:X40518	Human secreted protein 5' EST	381	242/242 (100%)	3.8e-49
patn:X40528	Human secreted protein 5' EST	399	280/286 (97%)	4.1e-57
gb:GENBANK- ID:AI557139 acc:AI5 57139	PT2.1_13_H11.r tumor2 Homo sapiens cDNA 3', mRNA sequence - Homo sapiens	1000	165/173 (95%)	5.5e-28

The GPCR7a protein encoded by SEQ ID NO:25 has 318 amino acid residues, and is presented using the one-letter code in Table 7B (SEQ ID NO:26). The SignalP, Psort and/or Hydropathy profile for GPCR7a predict that GPCR7a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence is coded for in the first 44 amino acids, *i.e.*, with a cleavage site at VRT-EH, between amino acids 54 and 55. This is typical of this type of membrane protein.

Table 7C. Encoded GPCR7 protein sequence (SEQ ID NO:26).
MMVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDIL ISTSSMPKMLAIFWFNSTTIQFDACLLQMFHSLSGMESTVLLAMAFDRYVAICHPLRHATVLTLPRTKIGVA AVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDDIRVNVVYGLIVIIISAIGLDSLLISFSYLLIL KTVLGLTREAAKAFGTCVSHVCAVFIFYVFFIGLSMVHRFSKRDSPLPVILANIYLLVPPVLNPIVYGVTKE IRQRILRLFHVATHASEP

The full amino acid sequence of the protein of the invention was found to have high homology to a number of polypeptides including the ones in the BLASTX alignments in Table 7D.

Table 7D. BLAST results for GPCR7a					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect

ptnr:SPTREMBL- ACC:088628	PUTATIVE G- PROTEIN COUPLED RECEPTOR RA1C - Rattus norvegicus (Rat)	320	183/305 (60%)	235/305 (77%)	1.5e- 98
patp:W01730	Human G-protein receptor HPRAJ70 - Homo sapiens	320	181/305 (59%)	235/305 (77%)	1.8e- 97
patp:Y11796	Human 5' EST secreted protein	21	20/20 (100%)	20/20 (100%)	1.3e- 05
gi 6532001 gb AAD27 596.2 AF121976_1 (AF121976)	odorant receptor S19 [Mus musculus]	339	158/280 (56%)	209/280 (74%)	4e-83

Patp results include those listed in Table 7C.

Table 7C. Patp alignments of GPCR7				
Sequences producing High-scoring Segment Pairs:				Smallest
		Reading Frame	High Score	Sum Prob. P (N)
patp:B43266	Human ORFX ORF3030 polypeptide sequence SE...	+2	927	2.6e-92
patp:B42796	Human ORFX ORF2560 polypeptide sequence SE..	+2	852	2.3e-84
patp:Y83390	Olfactory receptor protein OLF-5 - H. sapiens...	+2	727	4.1e-71
patp:Y54326	Amino acid sequence of marmot olfactory re..	+2	717	4.7e-70
patp:W75960	Human olfactory OLRC15 receptor - H. sapiens...	+2	713	1.2e-69
patp:R27868	Odorant receptor clone F5 - Rattus rattus, ..	+2	712	1.6e-69
patp:Y90874	Human G protein-coupled receptor GTAR14-5 ..	+2	697	6.2e-68

5 GPCR7b and c

In the present invention, the target sequence identified previously, Accession Number AC025249_A, was subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in PCR amplifications based on a library containing a wide range of cDNA species. The resulting two amplicons were gel purified, cloned and sequenced to high redundancy to provide the sequences reported below, which are designated GPCR7b (Accession Numbers AC025249_A1) and GPCR7c (AC025249_A_da3). The sequence of GPCR7b contains one amino acid difference from that of GPCR7a at position 247, wherein alanine is replaced by valine. The sequence of GPCR7c has one amino acid different at position 183, wherein leucine is replaced by proline.

GPCR7b

A novel GPCR nucleic acid was identified by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs, and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR7 nucleic acid of 969 nucleotides (also referred to as AC025249_A1) is shown in Table 7E. An open reading begins with an ATG initiation codon at nucleotides 7-9 and ends with a TAG codon at nucleotides 961-963. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 7E, and the start and stop codons are in bold letters.

Table 7E. GPCR7 Nucleotide Sequence (SEQ ID NO:27)

TTCTTCATGATGGTGGATCCCAATGGCAATGAATCCAGTGCTACATACTTCATCCTAATAGGCCTCCCTGGTTTAGAA
GAGGCTCAGTTCTGGTTGGCCCTTCCCATTGTGCTCCCTCTACCTTATTGCTGTGCTAGGTAAGTGAACATCATCTAC
ATTGTGCGGACTGAGCACAGCCTGCATGAGCCCATGTATATATTTCTTTGCATGCTTTCAGGCATTGACATCCTCATC
TCCACCTCATCCATGCCCAAAATGCTGGCCATCTTCTGGTTCAATCCACTACCATCCAGTTTGATGCTGTCTGTGTA
CAGATGTTTGCCATCCACTCCTTATCTGGCATGGAATCCACAGTGCTGCTGGCCATGGCTTTTGACCGCTATGTGGCC
ATCTGTCAACCACTGCGCCATGCCACAGTACTTACGTTGCCTCGTGTCAACCAAAATTGGTGTGGCTGCTGTGGTGGCG
GGGGCTGCACTGATGGCACCCTTCTGTCTTCATCAAGCAGTGCCCTTCTGCCGCTCCAATATCCTTTCCCATTCC
TACTGCCCACACCAAGATGTATGAAGCTGGCCTGTGATGATATCCGGGTCAATGTCTATGGCCTTATCGTCATC
ATCTCCGCCATTGGCCTGGACTCACTTCTCATCTCCTTCTCATATCTGCTTATTCTTAAGACTGTGTTGGGCTTGACA
CGTGAAGCCCAAGGCAAGGCATTGCGCACTTGGCTCTCTCATGTGTGTGCTGTGTTTATATTCTATGTACCTTTTCATT
GGATGTCCATGGTGCATCGCTTTAGCAAGCGCGTGACTCTCCACTGCCCGTCATCTGGCCAATATCTATCTGCTG
GTTCTCTGTGCTCAACCAATTGTCTATGGAGTGAAGACAAAGGAGATTGACAGCGCATCCTTCGACTTTTCCAT
GTGGCCACACAGCTTCAGAGCCCTAGGTGTCA

The GPCR7b protein encoded by SEQ ID NO:25 has 318 amino acid residues, and is presented using the one-letter code in Table 7B (SEQ ID NO:26). The SignalP, Psort and/or Hydropathy profile for GPCR7a predict that GPCR7a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence is coded for in the first 44 amino acids, i.e., with a cleavage site at VRT-EH, between amino acids 54 and 55. This is typical of this type of membrane protein.

Table 7F. Encoded GPCR7b protein sequence (SEQ ID NO:26).

MMVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDIL
ISTSSMPKMLAIFWFNSTTIQFDACLLQMFHLSLGMESTVLLAMAFDRYVAICHPLRHATVLTLPRTKIGVA
AVVRGAALMAPLPVFIKQLPFCRSNLSHSYCPHQDVMKLACDDIRNVVYGLIIVISAIGLDSLLISFSYLLIL
KTVLGLTREQAQAFGTCSHVCAVFIFYVPFGLSMVHRFSKRSDPLPVILANIYLLVPPVLNPIVYGVKTK
IRQIRILRLFHVATHASEP

The full amino acid sequence of GPCR7b was found to have 152 of 301 amino acid residues (50%) identical to, and 213 of 301 residues (70%) positive with, the 319 amino acid residue Putative G-Protein Coupled Receptor RA1C from *Rattus norvegicus* (SPTREMBL-ACC:O88628) (E value = $3.3e^{-81}$), and 142 of 295 amino acid residues (48%) identical to, and 199 of 295 residues (67%) positive with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (sptREMBL-Q9UKL2) (E value = $2.9e^{-75}$)

GPCR7b also has high homology to the following proteins found through BLASTX alignments, shown in Table 7G.

Table 7G. BLASTX results for GPCR7b				
Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
ptnr:SPTREMBL-ACC:O88628 PUTATIVE G-PROTEIN COUPLED RE.+1		981	6.6e-98	1
ptnr:SPTREMBL-ACC:Q9YH55 OLFACTORY RECEPTOR-LIKE PROTE.+1		816	2.0e-80	1
ptnr:SPTREMBL-ACC:Q9WVD9 MOR 3'BETA1 - Mus musculus (M.+1		812	5.3e-80	1
ptnr:SPTREMBL-ACC:Q9WU90 ODORANT RECEPTOR S19 - Mus mu.+1		799	1.3e-78	1
ptnr:SPTREMBL-ACC:Q9WVN5 MOR 5'BETA2 - Mus musculus (M.+1		777	2.7e-76	1
ptnr:SPTREMBL-ACC:Q9WVD8 MOR 3'BETA2 - Mus musculus (M.+1		769	1.9e-75	1
ptnr:SPTREMBL-ACC:Q9WU89 ODORANT RECEPTOR S18 - Mus mu.+1		769	1.9e-75	1
ptnr:SPTREMBL-ACC:Q9WVD7 MOR 3'BETA3 - Mus musculus (M.+1		764	6.5e-75	1
ptnr:SPTREMBL-ACC:Q9UKL2 OLFACTORY RECEPTOR HPFH1OR - .+1		760	1.7e-74	1
ptnr:SPTREMBL-ACC:Q9WVN6 MOR 5'BETA3 - Mus musculus (M.+1		756	4.6e-74	1
ptnr:SPTREMBL-ACC:Q9Y5P1 HOR 5'BETA3 - Homo sapiens (H.+1		723	1.4e-70	1
ptnr:SPTREMBL-ACC:Q9WVN4 MOR 5'BETA1 - Mus musculus (M.+1		707	7.1e-69	1
ptnr:SPTREMBL-ACC:Q9WU93 ODORANT RECEPTOR S46 - Mus mu.+1		701	3.1e-68	1
ptnr:SPTREMBL-ACC:Q9Y5P0 HOR 5'BETA1 - Homo sapiens (H.+1		630	1.0e-60	1
ptnr:SPTREMBL-ACC:Q9WU94 ODORANT RECEPTOR S50 - Mus mu.+1		578	3.3e-55	1

GPCR7c

The disclosed novel GPCR7c nucleic acid of 968 nucleotides (also referred to as AC025249_Ada3) is shown in Table 7H. An open reading begins with an ATG initiation codon at nucleotides 7-9 and ends with a TAG codon at nucleotides 961-963. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 7H, and the start and stop codons are in bold letters.

Table 7H. GPCR7 Nucleotide Sequence (SEQ ID NO:29)

<p><u>TTCTTCATGATGGTGGATCCCAATGGCAATGAATCCAGTGCTACATACTTCATCCTAATAGGCCTCCTGGTTAGAA</u> GAGGCTCAGTTCTGGTTGGCCTTCCCATTGTGCTCCTCTACCTTATTGCTGTGCTAGGTAACCTTGACAATCATCTAC ATTGTGCGGACTGAGCACAGCCTGCATGAGCCCATGTATATATTTCTTTGCATGCTTTTCAGGCATTGACATCCTCATC TCCACCTCATCCATGCCCAAAATGCTGGCCATCTTCTGGTTCAATTCACCTACCATCCAGTTTGATGCTTTGCTGCTA CAGATGTTTGCCATCCACTCCTTATCTGGCATGGAATCCACAGTGCTGCTGGCCATGGCTTTTGACCGCTATGTGGCC ATCTGTCACCCACTGCGCCATGCCACAGTACTTACGTTGCCTCGTGTCAACAAATTGGTGTGGCTGCTGTGGTGCGG GGGGCTGCACTGATGGCACCCCTTCTGTCTTCATCAAGCAGCTGCCCTTCTGCGCTCCAATATCCTTTCCCATTC TACTGCCACACCAAGATGTCATGAAGCTGGCCTGTGATGATATCCGGGTCAATGTGCTCTATGGCCTTATCGTCATC ATCTCCGCTATTGGCCTGGACTCACTTCTCATCTCCTTCTCATATCTGCTTATTCTTAAGACTGTGTGGGCTTGACA CGTGAAGCCCAAGGCATTGTCACCTTGCCTCTCATGTGTGTGCTGTGTTTATATTCTATGTACCTTTTCATT GGATTGTCCATGGTGCATCGCTTTAGCAAGCGCGTGACTCTCCACTGCCCGTCATCTTGCCAATATCTATCTGCTG</p>

GTTCTCTCTGTGCTCAACCAATTGTCTATGGAGTGAAGACAAAGGAGATTGACAGCGCATCCTTCGACTTTTCCAT
GTGGCCACACACGCTTCAGAGCCCCAGGTGTA

The GPCR7c protein encoded by SEQ ID NO:30 has 318 amino acid residues, and is presented using the one-letter code in Table 7I (SEQ ID NO:30). The SignalP, Psort and/or Hydropathy profile for GPCR7a predict that GPCR7a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence is coded for in the first 44 amino acids, *i.e.*, with a cleavage site at VRT-EH, between amino acids 54 and 55. This is typical of this type of membrane protein.

Table 7I. Encoded GPCR7c protein sequence (SEQ ID NO:30).

MMVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDIL
ISTSSMPKMLAIFWFNSTTIQFDACLLQMFHLSLGMESTVLLAMAFDRYVAICHPLRHATVLTLPRTKIGVA
AVVRGAALMAPLPVFIKQLPFCRSNLSHSYCPHQDVMKLACDDIRVNVVYGLIVIIISAIGLDSLLISFSYLLIL
KTVLGLTREAQAKAFGTCVSHVCAVFIYVVPFGLSMVHRFSKRDSPLPVILANIYLLVPPVLPNPIVYGVKTKKE
IRQRILRLRFHVATHASEP

Homologous proteins to GPCR7c were searched for using BLASTX. Some of the alignments are included in Table 7J.

Table 7J. BLAST results for GPCR7c

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ptnr:SPTREMBL- ACC:088628	PUTATIVE G- PROTEIN COUPLED RECEPTOR RA1C - Rattus norvegicus (Rat)	320	183/305 (60%)	235/305 (77%)	1.5e- 98
ptnr:SPTREMBL- ACC:Q9YH55	OLFACTORY RECEPTOR-LIKE PROTEIN COR3'BETA - Gallus gallus s	319	152/301 (50%)	213/301 (70%)	3.3e- 81
ptnr:SPTREMBL- ACC:Q9UKL2	OLFACTORY RECEPTOR HPFH1OR - Homo sapiens	312	142/295 (48%)	199/295 (67%)	2.9e- 75

GPCR7c also has high homology to the following proteins found through BLASTX alignments, shown in Table 7K.

Table 7K. BLASTX results for GPCR7b

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
ptnr:SPTREMBL-ACC:088628 PUTATIVE G-PROTEIN COUPLED RE.	+1	981	6.8e-98	1
ptnr:SPTREMBL-ACC:Q9YH55 OLFACTORY RECEPTOR-LIKE PROTE.	+1	816	2.1e-80	1
ptnr:SPTREMBL-ACC:Q9WVD9 MOR 3'BETA1 - Mus musculus (M.)	+1	812	5.5e-80	1
ptnr:SPTREMBL-ACC:Q9WU90 ODORANT RECEPTOR S19 - Mus mu.	+1	799	1.3e-78	1

Possible SNPs found for GPCR7c are listed in Table 7L.

Table 7L: SNPs			
Consensus Position	Depth	Base Change	PAF
446	18	A>-	0.111
513	17	T>C	0.118
555	16	T>C	0.125
827	9	A>G	0.333

The disclosed GPCR7 protein has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 7N. The GPCR7 protein has significant identity to olfactory receptor (OR) proteins (Table 7M):

Table 7M. BLAST results for GPCR7					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 3420759 gb AAD12761.1 (AF079864)	putative G-protein coupled receptor RA1c [Rattus norvegicus]	320	173/299 (57%)	221/299 (73%)	3e-88
gi 11875778 gb AAG40776.1 AF311306_1 (AF311306)	prostate specific G-protein coupled receptor; PSGR [Homo sapiens]	320	173/299 (57%)	223/299 (73%)	5e-88
gi 11908213 gb AAG41678.1 (AF137396)	HOR5'Beta12 [Homo sapiens]	312	157/304 (51%)	205/304 (66%)	4e-76
gi 11908220 gb AAG41684.1 (AF133300)	MOR 3'Beta4 [Mus musculus]	319	156/308 (50%)	207/308 (66%)	6e-73
gi 11908214 gb AAG41679.1 (AF137396)	HOR5'Beta11 [Homo sapiens]	314	143/310 (46%)	207/310 (66%)	2e-69

This information is presented graphically in the multiple sequence alignment given in Table 7E (with GPCR7 being shown on line 1) as a ClustalW analysis comparing GPCR7 with related protein sequences.

Table 7E. Information for the ClustalW proteins:

- 1) GPCR7a (SEQ ID NO:29)
- 2) GPCR7b
- 2) gi|3420759|gb|AAD12761.1| (AF079864) putative G-protein coupled receptor RA1c [Rattus norvegicus]
- 3) gi|11875778|gb|AAG40776.1|AF311306_1 (AF311306) prostate specific G-protein coupled receptor; PSGR [Homo sapiens]

- 4) gi|11908213|gb|AAG41678.1| (AF137396) HOR5'Beta12 [Homo sapiens]
 5) gi|11908220|gb|AAG41684.1| (AF133300) MOR 3'Beta4 [Mus musculus]
 6) gi|11908214|gb|AAG41679.1| (AF137396) HOR5'Beta11 [Homo sapiens]

5

	10	20	30	40	50	60
GPCR7a	MMVDPNGNESSATY	ELIGPGLEEAQFWLAFPLCSYLTAVLGNLTIIYIVRTEHSLHE				
GPCR7b	MMVDPNGNESSATY	ELIGPGLEEAQFWLAFPLCSYLTAVLGNLTIIYIVRTEHSLHE				
gi 3420759	----	MSSCNFTHATFMILIGIPGLEEAHFWGFPILLSYVAVALFGNCIVVFIVRTERSILHA				
gi 11875778	----	MSSCNFTHATFMILIGIPGLEEAHFWGFPILLSYVAVAMFGNCIVVFIVRTERSILHA				
gi 11908213	----	MGLFNVTHPAFFELITIGIPGLESCHSWLSGPLGVVAVAVGGNTVILQAVVEPSLHE				
gi 11908220	----	MATSNSSSTIVSSSTYLTGIPCYEEFHHWSIIPCFYVAVGTGNCMLHHIVRTDPRLHE				
gi 11908214	----	MLGLNGTPFPQATLQITGIPGLOTGLTWALIFGILYVISTVGNLSIHTLVFWEPALHQ				
	70	80	90	100	110	120
GPCR7a	PMYIFLOMLSGIDILHSTSSMPKMLAFWFNSTTIOFACLIOMFAHSLSGMESTVLLA					
GPCR7b	PMYIFLOMLSGIDILHSTSSMPKMLAFWFNSTTIOFACLIOMFAHSLSGMESTVLLA					
gi 3420759	PMYIFLOMLAIDIALSTSTMPKMLAFWFNSREITPACLAQMFHIALSAESTVLLA					
gi 11875778	PMYIFLOMLAIDIALSTSTMPKMLAFWFNSREITPACLAQMFHIALSAESTVLLA					
gi 11908213	PMYIFLSMLSFSDVAISMATPTVLRTECLMARNITPACLIOMFLIHFFSMESGILLA					
gi 11908220	PMYIFLAMLSLIDMAMSLFTMMSTFRWLWSISREIQFNICVVMFLIHTFTSSVLLA					
gi 11908214	PMYIFLSMLALNLDGVSFSTLETVISTECFNYNHAFNACLVCMEFHTFTSFMESGILLA					
	130	140	150	160	170	180
GPCR7a	MAFDRYVAICHPLRHAATVLTLPRTKICVAAVVRGAALMAPLEVFTKLPFCRSNLSHS					
GPCR7b	MAFDRYVAICHPLRHAATVLTLPRTKICVAAVVRGAALMAPLEVFTKLPFCRSNLSHS					
gi 3420759	MAFDRYVAICHPLRHAATVLTLPRTKICVAAVVRGAALMAPLEVFTKLPFCRSNLSHS					
gi 11875778	MAFDRYVAICHPLRHAATVLTLPRTKICVAAVVRGAALMAPLEVFTKLPFCRSNLSHS					
gi 11908213	MSFDRYVAICDPLRYATVLTTEVTAAAGLGAARSFITLFFLEFLIKKLPFCRSNLSHS					
gi 11908220	MALDRYVAICHPLRYATILTLPKLTAKICTAALLRSSILTIPIIARLAFFPFCGSIVLSHS					
gi 11908214	MSLDRYVAICHPLRYATVLTTHNRILAMCGILTKSFTTLPFFEFVVKKLPFCCKGNVLSHS					
	190	200	210	220	230	240
GPCR7a	YCFHQDVMKLACDDIRVNVVYGLIVITSAIGLDSLLISFSYLLILIKTVIGET-REAOAKA					
GPCR7b	YCFHQDVMKLACDDIRVNVVYGLIVITSAIGLDSLLISFSYLLILIKTVIGET-REAOAKA					
gi 3420759	YCFHQDVMKLAYDTLPLNVVYGLITAILVMGVDMVFISFSYFLIIRAVLQPSKSERAKA					
gi 11875778	YCFHQDVMKLAYDTLPLNVVYGLITAILVMGVDMVFISFSYFLIIRAVLQPSKSERAKA					
gi 11908213	YCFHQDVMKLACADISTNSYGLFVLVSTFGMDLFFIFLSYVLILDSVMATASREERLKA					
gi 11908220	YCFHQDVMKLACADIRPNVYGLIVITLLWCHDSLGTFVSVVLILHSVLIKIASRECRILKA					
gi 11908214	YCFHPDVMKYACGDIVHVNBYGLIWIIFTYGLDSTFLLSYALILRAMLVVLSQERLKA					
	250	260	270	280	290	300
GPCR7a	FGTCVSHVCAVFIYVVFIFGLSVVHRFSKRRDSPLPVILANIVLLVPPVLPNPIYGVKTK					
GPCR7b	FGTCVSHVCAVFIYVVFIFGLSVVHRFSKRRDSPLPVILANIVLLVPPVLPNPIYGVKTK					
gi 3420759	FGTCVSHIGVVLAFYVVPFIFGLSVVHRFGNSLDPIVHVLMGVDVYLLPPVLPNPIYGAATK					
gi 11875778	FGTCVSHIGVVLAFYVVPFIFGLSVVHRFGNSLDPIVHVLMGVDVYLLPPVLPNPIYGAATK					
gi 11908213	LNTCVSHILAVLAFYVVPFIFGLSVVHRFGKHVPCYTHVFMNSVYLEVPPVLPNPIYSAATK					
gi 11908220	LNTCVSHICAVLILYVVPFIFGLSVVHRFAKHSSPLIHFMAHIVLLVPPVLPNPIYSVKTK					
gi 11908214	LNTCVSHICAVLAFYVVPFIFGLSVVHRFWSAPPVHVHMSNVYLEVPPVLPNPIYSVKTK					
	310	320				
GPCR7a	EIRQRILRLSHVATHASEP----					
GPCR7b	EIRQRILRLSHVATHASEP----					
gi 3420759	QIRTRVLAMKISCDKDIEAGGNT					
gi 11875778	QIRTRVLAMKISCDKDLQAVGGK					
gi 11908213	EIRRAFRMHHIKI-----					
gi 11908220	QIREGIIHLCSPKISSITM----					
gi 11908214	EIRKGIILKFHKESQA-----					

The presence of identifiable domains in GPCR7 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the

Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR7 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 7F with the statistics and domain description. The results indicate that this protein contains homology to the following protein domains (as defined by Interpro) at the indicated positions: domain name 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family). This indicates that the sequence of GPCR7 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

Table 70. DOMAIN results for GPCR7

CD-Length = 254 residues, 100.0% aligned
Score = 77.4 bits (189), Expect = 1e-15

		10	20	30	40	50	60
15	GPCR7	GNLITTYIVRTEHS	SH-EMYIFLCM	SGITLITSTSSMPKM	AIFWFN-STTQFDACL		
	Consensus 7tm 1 domain	GNLLVTLVILRTKKLR	TETNIFLLNLQVADLLFLLILFEWALYYLVGG--DWYFGDALC				
		70	80	90	100	110	120
20	GPCR7	LQMPATHSTSCMESTVLL	AMAFDRYVAICHPLRHATVLLPRTK	GVAAVVRGAALMA			
	Consensus 7tm 1 domain	KLVGALFVWNGYASITLLTAISIDRYEAIIVHPLRRTT	PRRAKVL	LLLVVVLALIS			
		130	140	150	160	170	180
25	GPCR7	PLPVFIKQLPFCRSNI	LSHSYCLHQDVMKLACDDIRVNVVYGLIV	ISAIGLDSLI			
	Consensus 7tm 1 domain	LPPQLFSWRTVEEGNT	TVCLIDFPESVK	RSYVLLSTLVGFLPLIVLV			
		190	200	210	220	230	240
30	GPCR7	ISFSYLLILK					
	Consensus 7tm 1 domain	CYTRILRTLRKSAR					
		250	260	270	280	290	300
35	GPCR7						
	Consensus 7tm 1 domain						
		310	320	330	340	350	360
40	GPCR7	TVLGLTREAQA	KAFGTCVSHVCAVFF	FFVVP	IGLSMV		
	Consensus 7tm 1 domain	SQRSIKRRSSERKA	AKMLLVVVVFVLCWPEPHIVLLDST				
		370	380	390			
45	GPCR7	HRFSKR	RDSPLPVITANIYLLVPPVLNP	IV			
	Consensus 7tm 1 domain	CLLSIWR	VLPETALLTLWLAYVNSCLNP	IV			

The similarity information for the GPCR7 protein and nucleic acid disclosed herein suggest that GPCR7 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic

applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon. The novel nucleic acid encoding GPCR7, and the GPCR7 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in used in the treatment of infections such as bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The disclosed GPCR7 polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium,

dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

GPCR8

GPCR8a

A novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR8a nucleic acid of 985 nucleotides (also referred to as AC025249) is shown in Table 8C. An open reading begins with an ATG initiation codon at nucleotides 5-7 and ends with a TGA codon at nucleotides 977-979. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 8C, and the start and stop codons are in bold letters.

Table 8C. GPCR8 Nucleotide Sequence (SEQ ID NO:32)

TG	TG	AT	G	C	T	G	G	G	T	C	C	A	G	C	T	T	A	A	C	C	A	C	A	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	G	A	C	T	G	C	T	T	T	G	G	C	T	A	T	C	T	C	A	C	T	G	A	G	T	G	C	C	A	T	G	T	A	C	A	T	C	G	
CA	AT	C	T	T	C	A	C	A	T	C	T	T	T	G	G	C	T	A	T	C	A	C	T	A	C	A	G	A	A	T	T	C	A	C	A	G	A	A	T	T	C	A	C	G	C	C	T	G	T	T	A	G	G	A	A	C	C	A	C	C	A	T	C	A	T	C	G																								
TG	AC	T	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G	C	C	T	C	C	T	T	G	G	G	T	A	T	C	C	C	A	G	A	A	C	A	C	C	A	T	C	A	T	C	G																																									
TG	AT	G	C	T	T	C	A	C	A	T	G	G	A	A	C	C	C	T	G																																																																								

The GPCR8 protein encoded by SEQ ID NO:32 has 324 amino acid residues, and is presented using the one-letter code in Table 8B (SEQ ID NO:33). The SignalP, Psort and/or Hydropathy profile for GPCR8a predict that GPCR8a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence with a cleavage site at the slash in the sequence VTA-IW, between amino acids 52 and 53. This is typical of this type of membrane protein.

Table 8B. Encoded GPCR8 protein sequence (SEQ ID NO:33).	
MLGPAYNHTMETPASFLLVGIPGLQSSHLWLAI	SLSAM YITALLGNTLIVTAIWMDSTRHEP
MYCFLCVLA	AVDIV
MASSVVPKMVSIFCSGDSSISFSACFTQMF	FFVHLATAVETGLLLTMAFD
RYVAICKPLHYKRILTPQV	MLGMSMAV
TIRAVTFMTPLSWMMNHL	PFCGSNVVVHSYCKHIALARLACADPVP
SSLYSLIGSSLMVGS	DAFIAASYILILRA
VFDLSSKTAQLKALSTCGSHVGMALY	YLPGMASIYAANL
QD	I VPLHTQVLLADLYV
IIPATLNPII	YGMRTKQL
LEGIWSYLMHFL	FDHSNLGS

The full amino acid sequence of the protein of the invention was found to have 146 of 306 amino acid residues (47%) identical to, and 202 of 306 residues (66%) positive with, the 321 amino acid residue Odorant Receptor S18 from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU89) (E value = 8.2e⁻⁷³), and 124 of 297 amino acid residues (41%) identical to, and 196 of 297 residues (65%) positive with, the 320 amino acid residue G-protein coupled prostate tissue receptor designated HPRAJ70 from *Homo sapiens* (patp:W56641) (E value = 3.4e⁻⁶⁶).

GPCR8b

A novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR8a nucleic acid of 980 nucleotides (also referred to as AC025249_B) is shown in Table 8A. An open reading begins with an ATG initiation codon at nucleotides 3-5 and ends with a TGA codon at nucleotides 975-977. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 8A, and the start and stop codons are in bold letters.

Table 8A. GPCR8 Nucleotide Sequence (SEQ ID NO:31)	
<u>TG</u> ATG <u>CTGGGTCCAGCTTACAAC</u>	<u>CACACAATGGA</u> <u>AACCCCTGCCTCCTTCCTCCTTGTGGGTATCC</u>
<u>CAGGACTGCA</u>	

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ATCTTCACATCTTTGGCTGGCTATCTCACTGAGTGCCATGTACATCACAGCCCTGTTAGGAAACACCCTCATCGTG
ACTGCAATCTGGATGGATTCCACTCGGCATGAGCCCATGTATTGCTTTCTGTGTGTTCTGGCTGCTGTGGACATGT
TTATGGCTCCTCCGTGGTACCCAAGATGGTGAGCATCTTCTGCTCGGGAGACAGCTCCATCAGCTTTAGTGCTTG
TTTCACTCAGATGTTTTTTGTCCACTTAGCCACAGCTGTGGAGACGGGGCTGCTGCTGACCATGGCTTTTGACCGC
TATGTAGCCATCTGCAAGCCTCTACACTACAAGAGAATTCTCACGCCTCAAGTGATGCTGGGAATGAGTATGGCCG
TCACCATCAGAGCTGTACATTCATGACTCCACTGAGTTGGATGATGAATCATCTACCTTTCTGTGGCTCCAATGT
GGTTGTCCACTCCTACTGTAAGCACATAGCTTTGGCCAGGTTAGCATGTGCTGACCCCGTGCCAGCAGTCTCTAC
AGTCTGATTGGTTCCCTCTCTTATGGTGGGCTCTGATGTGGCCTTCATTGCTGCCTCCTATATCTTAATCTCAGGG
CAGTATTTGATCTCTCCTCAAAGACTGCTCAGTTGAAAGCATTAAAGCACATGTGGCTCCCATGTGGGGGTTATGGC
TTTGTACTATCTACCTGGGATGGCATCCATCTATGCGGCTGGTTGGGGCAGGATATAGTGCCCTTGACACACCCAA
GTGCTGCTAGCTGACCTGTACGTGATCATCCAGCCACTTTAAATCCCATCATCTATGGCATGAGGACCAACAAT
TGCTGGAGGGAATATGGAGTTATCTGATGCACCTCTTGTGACCACTCCAACCTGGGTTTCATGAACA

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The disclosed nucleic acid sequence has 537 of 858 bases (62%) identical to a *Mus musculus* odorant receptor S19 gene, complete cds:(GENBANK-ID:AF121976|acc:AF121976) (E value = $3.5e^{-41}$).

The GPCR8 protein encoded by SEQ ID NO:31 has 324 amino acid residues, and is presented using the one-letter code in Table 8B (SEQ ID NO:32). The SignalP, Psort and/or Hydropathy profile for GPCR8a predict that GPCR8a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence with a cleavage site at the slash in the sequence VTA-IW, between amino acids 52 and 53. This is typical of this type of membrane protein.

Table 8B. Encoded GPCR8 protein sequence (SEQ ID NO:32).

```

MLGPAYNHTMETPASFLVGIPLQSSHLWLAI SLSAMYI IALLGNTI I VTAIWMDSTRHEPMYCFLCVLAAVDIV
MASSVVPKMVSIFCSGDSSISFSACFTQMFFVHLATAVETGLLLTMAFDTRYVAICKPLHYKRILTPQVMLGMSMAI
TIRAI IAITPLSWMVSHLPFCGSNVVHSYCEHIALARLACADPVPSLSYSLIGSSLMVGSVDVAFIAASYILILKA
VFGLSSKTAQLKALSTCGSHVGVMALYLPGMASIYAAWLQDQDVVPLHTQVLLADLYVIIIPATLNP I IYGMRTKQL
RERIWSYLMHVLFDHNSNLGS

```

The full amino acid sequence of the protein of the invention was found to have 146 of 306 amino acid residues (47%) identical to, and 202 of 306 residues (66%) positive with, the 321 amino acid residue Odorant Receptor S18 from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU89) (E value = $8.2e^{-73}$), and 124 of 297 amino acid residues (41%) identical to, and 196 of 297 residues (65%) positive with, the 320 amino acid residue G-protein coupled prostate tissue receptor designated HPRAJ70 from *Homo sapiens* (patp:W56641) (E value = $3.4e^{-66}$).

Patp results include those listed in Table 8C.

Table 8C. Patp alignments of GPCR8

Sequences producing High-scoring Segment Pairs:			Reading Frame	High Score	Smallest Sum Prob. P (N)
patp:B43266	Human ORFX ORF3030 polypeptide sequence SE.	..	+1	878	4.1e-87
patp:Y54326	Amino acid sequence of marmot olfactory receptor.	..	+1	814	2.5e-80
patp:B42796	Human ORFX ORF2560 polypeptide sequence SE..	..	+1	807	1.4e-79
patp:Y83390	Olfactory receptor protein OLF-5 - H, sapiens..	..	+1	652	3.6e-63

For example, a BLAST against Y54326, a 237 amino acid olfactory receptor protein from *Marmota marmota*, produced 156/237 (65%) identity, and 188/237 (79%) positives (E = 2.5e-80), with long segments of amino acid identity, as shown in Table 8D. WO 99/67282.

Table 8D. Alignment of GPCR8 with Y54326 (SEQ ID NO:88)

	10	20	30	40	50	60	
Y54326						PMV 3
GPCR8	MEKSNVSSVYGFI	LVGFS	DRPKLE	MVLF	TVNFIL	YSVAVLGNSTI	ILVCILDSQLHTPMV 60
	70	80	90	100	110	120	
Y54326	IFLNL	SFLD	SFTSS	IPQL	LNLS	GRD	KTISYVGC
GPCR8	FFLAN	SFLD	CFST	CI	PCML	VNLW	GDKTISCAGC
	130	140	150	160	170	180	
Y54326	DRFVAV	CKPLH	YIV	IMSS	RCL	GLVSV	AWGCC
GPCR8	DSYAA	VCKPL	RVLV	IMH	LQL	CLGL	MAAWG
	190	200	210	220	230	240	
Y54326	MPAL	IRMAC	VNTVA	IEG	TVFL	AVG	IVLSPI
GPCR8	KPAL	IRMAC	VNTVA	IEG	TVFL	AVG	IVLSPI
	250	260	270	280	290	300	
Y54326	CGSH	LTIV	SLFY	GNII	YMY	MPG	SRSSQ
GPCR8	CGSH	LTIV	SLFY	GNII	YMY	MPG	SRSSQ

Further BLAST analysis produced the significant results listed in Table 8E. The disclosed GPCR8 protein (SEQ ID NO:31) has good identity with a number of olfactory receptor proteins.

Table 8E. BLAST results for GPCR8

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Gi 4826521 emb CAB42853.1 (AL035402, dJ88J8.1) (AJ302594-99) (AJ302600-01)	novel 7 tm receptor protein (rhodopsin fam., OR-like) (hs6M1-15) <i>Homo sapiens</i>	320	177/305 (58%)	226/305 (74%)	5e-92

Gi 12054431 emb CAC20523.1 (AJ302603)	OR <i>Homo sapiens</i>	320	176/305 (57%)	226/305 (73%)	1e-91
Gi 12054429 emb CAC20522.1 (AJ302602)	OR <i>Homo sapiens</i>	320	177/305 (58%)	225/305 (73%)	1e-91
Gi 6679170 ref NP_032788.1	OR 15 (OR3) <i>Mus musculus</i>	312	166/307 (54%)	211/307 (68%)	2e-81
Gi 12231029 sp Q15062 O2H3_HUMAN	OR 2H3 <i>Homo sapiens</i>	316	163/306 (53%)	208/306 (67%)	5e-81

This information is presented graphically in the multiple sequence alignment given in Table 8F (with GPCR8 being shown on line 1) as a ClustalW analysis comparing GPCR8 with related protein sequences.

Table 8F. Information for the ClustalW proteins:

- 1) GPCR8 (SEQ ID NO:31)
- 2) gi|4826521|emb|CAB42853.1| dJ88J8.1 (novel 7 TM receptor (rhodopsin family) (OR like) protein) (hs6M1-15)) Homo sapiens (SEQ ID NO:59)
- 3) gi|12054431|emb|CAC20523.1| olfactory receptor Homo sapiens (SEQ ID NO:60)
- 4) gi|12054429|emb|CAC20522.1| olfactory receptor Homo sapiens (SEQ ID NO:61)
- 5) gi|6679170|ref|NP_032788.1| olfactory receptor 15 Mus musculus (SEQ ID NO:58)
- 6) gi|12231029|sp|Q15062|O2H3_HUMAN OR 2H3 (OR-LIKE PRT FAT11) (SEQ ID NO:56)

GPCR8	MEKSNVSSVYGFILVGFSDRPKLEMMFTNFILNSVAITGNSTIILVCHLDSQLHTPMY
gi 4826521	MDQSNYSSEHGFIILGFSPHKKMEMILSGVVAIFVLIITLVGNTAIIILASLDSQLHTPMY
gi 12054431	MDQSNYSSEHGFIILGFSPHKKMEMILSGVVAIFVLIITLVGNTAIIILASLDSQLHTPMY
gi 12054429	MDQSNYSSEHGFIILGFSPHKKMEMILSGVVAIFVLIITLVGNTAIIILASLDSQLHTPMY
gi 6679170	MEVDSNSSSGTIFILGVSDHPHLEIIFFAVELASVLIITLVGNLTIIILSLDARLHTPMY
gi 12231029	--MDNQSSTPGFIILGFSEHFGERTLFVVVFTSYLIITLVGNLTIIILSLDPRKLSPMY
GPCR8	FFLRNLSPDLDCFTTSIIIPQMLVNLWGPDKTISYGCCTQLYVVMWLGSECELLAVMSY
gi 4826521	FFLRNLSPDLDCFTTSIIIPQMLVNLWGPDKTISYGCCTQLYVVMWLGSECELLAVMSY
gi 12054431	FFLRNLSPDLDCFTTSIIIPQMLVNLWGPDKTISYGCCTQLYVVMWLGSECELLAVMSY
gi 12054429	FFLRNLSPDLDCFTTSIIIPQMLVNLWGPDKTISYGCCTQLYVVMWLGSECELLAVMSY
gi 6679170	FFLRNLSPDLDCFTTSIIIPQMLVNLWGPDKTISYGCCTQLYVVMWLGSECELLAVMSY
gi 12231029	FFLRNLSPDLDCFTTSIIIPQMLVNLWGPKKTISETDCSMDIETELSLGTECELLTVMAR
GPCR8	DSYAATCKPLHYFVVMNPHLCMKMIIMTWSISLANSVVLECTLINLETCCGNNIEDHFLCE
gi 4826521	DRFTATCKPLHYFVVMNPHLCMKMIIMTWSISLANSVVLECTLINLETCCGNNIEDHFLCE
gi 12054431	DRFTATCKPLHYFVVMNPHLCMKMIIMTWSISLANSVVLECTLINLETCCGNNIEDHFLCE
gi 12054429	DRFTATCKPLHYFVVMNPHLCMKMIIMTWSISLANSVVLECTLINLETCCGNNIEDHFLCE
gi 6679170	DRYVAVCPPLHYMTVMNFRLCWGLAAISWLGGLGNSVLOSTFTLOLEFCGRKVDNFLCE
gi 12231029	DRYVAVCPPLHYATTHPRLCWGLASMAVITGLVESVVQTPSTLLEFCPRQVDDFVCE
GPCR8	KPALRKACQDITVEMSVFALGIIIVLTPLILILISYGYIAKAVLRITKSKASQKAMNT
gi 4826521	LPALVKKACQDITVEMSVFALGIIIVLTPLILILISYGYIAKAVLRITKSKASQKAMNT
gi 12054431	LPALVKKACQDITVEMSVFALGIIIVLTPLILILISYGYIAKAVLRITKSKASQKAMNT
gi 12054429	LPALVKKACQDITVEMSVFALGIIIVLTPLILILISYGYIAKAVLRITKSKASQKAMNT
gi 6679170	VPAMIKACQDITSLNEAVINGVCTFFTVVPVSILISYCFIAQAVMKIRISVEGRKAFNT
gi 12231029	VPALRLACQDITSYNEIQVAVASVFIIVVPLSLILISYCAITWAVLRINSKAGGRKAFGT
GPCR8	CGSHLTIVVSMFYGIIIMYMLQPGNRASDQGKFLTLFYIVITPSLNPLIYTLRNKMDKA
gi 4826521	CGSHLTIVVSMFYGIIIMYMLQPGNRASDQGKFLTLFYIVITPSLNPLIYTLRNKMDKA
gi 12054431	CGSHLTIVVSMFYGIIIMYMLQPGNRASDQGKFLTLFYIVITPSLNPLIYTLRNKMDKA
gi 12054429	CGSHLTIVVSMFYGIIIMYMLQPGNRASDQGKFLTLFYIVITPSLNPLIYTLRNKMDKA

gi 6679170	CVSHLVVVFIFVCSATYGYLPAKSSNQSGKFISLFYSVNTPMVNPLIYTLRNKEKGA
gi 12231029	QSSHLTVVFIFVSSVFAVYLOPKNPYAQGRGKEFGLFYAVGTPLNPLIYTLRNKEVTRA
	310 320
GPCR8	LKKVLEGRQMLEKYDKL---
gi 4826521	LKKLMRFHHKSTIKRNCKS
gi 12054431	LKKLMRFHHKSTIKRNCKS
gi 12054429	LKKLMRFHHKSTIKRNCKS
gi 6679170	EGRLGKGRG-----AS--
gi 12231029	FRRLGKERDRESWRAA--

The presence of identifiable domains in GPCR8 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR8 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 8G with the statistics and domain description. The results indicate that GPCR8 contains the 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family) domain (as defined by Interpro) at amino acid positions residues 41-170. This indicates that the sequence of GPCR8 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

Table 8G. DOMAIN results for GPCR8

gnl Pfam pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family)
Length = 377 (SEQ ID NO:45).Score = 85.9 bits (211), Expect = 3e-18
GPCR8
TM7
GPCR8
TM7

The similarity information for the GPCR8 protein and nucleic acid disclosed herein suggest that GPCR8 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid

useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon. The novel nucleic acid encoding GPCR8, and the GPCR8 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

The disclosed GPCR8 nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer; immune response; AIDS; asthma; Crohn's disease; multiple sclerosis; and treatment of Albright hereditary osteodystrophy and/or other pathologies and disorders. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer; immune response; AIDS; asthma; Crohn's disease; multiple sclerosis; and treatment of Albright hereditary osteodystrophy. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immuno-specifically to the novel GPCR8 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR8 Antibodies" section below. For example the disclosed GPCR8 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR8 epitope is from about amino acids 160 to 180. In another embodiment, a GPCR8 epitope is from about amino acids 225 to 240. In additional embodiments, GPCR8 epitopes are from amino acids 260 to 275 and from amino acids 290 to 330. These novel proteins can also be used to develop assay system for functional analysis.

GPCR9

A novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probe or homolog, run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for

apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR9 nucleic acid of 946 nucleotides (also referred to as 6-L-19-B) is shown in Table 9A. An open reading begins with an ATG initiation codon at nucleotides 5-7 and ends with a TAA codon at nucleotides 932-934. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 9A, and the start and stop codons are in bold letters.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 563 of 902 bases (62%) identical to (E= 8.5e-47) a *Mus musculus* gene for odorant receptor A16 (GENBANK-ID: GENBANK-ID:AB030896|acc:AB030896). In a search of sequence databases, partial matches (353 of 373 bases, 94% identical) were also identified with the nucleotides 1- 372 of GPCR9 identical with nucleotides 306-678 of a *Homo sapiens* GPCR EST (GENBANK-ID: GENBANK-ID: AW182678|acc:AW182678; xj45d11.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2660181 3' similar to TR:Q9Z1V0 Q9Z1V0 OLFACTORY RECEPTOR C6). This 94% match (E=1.1e-69) between regions of the public sequence and regions of the present invention (gene) suggests that the present invention (gene) could be a splice variant of the public GPCR EST (partial mRNA). This also supports identification of GPCR9 as a GPCR. In a search of sequence databases, partial matches (94 of 100 bases, 94% identical) of nucleotides 893-794 of GPCR9 with nucleotides 251-348 of a *Homo sapiens* GPCR EST (GENBANK-ID: AA206680|acc:AA206680: zq51c11.r1 Stratagene neuroepithelium (#937231) Homo sapiens cDNA clone IMAGE:645140 5' similar to contains L1.b2 L1 repetitive element). This 94% match between nucleotides of the public sequence and nucleotides of the GPCR9 sequence suggests that GPCR9 may be a splice variant of the public GPCR EST (partial mRNA).

Table 9A. GPCR9 Nucleotide Sequence (SEQ ID NO:32)

GTTCATGGAATAGGAATATTGTCACCTGTCTTTATTCTCCTGGGACTTTCTCAAACAAGAACATTGAA
GTTTTTGGTTTGTATTATTGTATTTGCTACATTGCTATTGGATGGAAACTTCATCATAATGATTT
CTATCATGTACATTTGGCTAATTGACCAACCCATGTATTTCTTCCTTAATTACCTCGCACTCTCAGATCT
TTGCTACATATCCACTGTGGCCCCAAGCTAATGATTGACCTACTAACAGAAAGGAAGATCGTTTCCTAT
AATAACTGCATGATACAGCTATTTATCACTCACTTCCTGGAGACATTGAGATCTTCATACTCAAAGCAA
TGGCCTATGACCACTACATAGCCATCTGCAAGCACCTGCACTACACCATCATCAGACCAAGCAAAGCTG
TAACACCATCATCATAGCTTGTGTACTGGGGGATTATACACTCTGCCAGTCAGTTTCTTCTTACCATC
TTCTTACCGTTCTGTGGTCTTAATGAGATAGATCAGTACTTCTGCTATGTGTATCCTCTGCTGAAGTTGG
CTCGCATTGATATATACAGAATTGGTTTCTTGGTAATTGTTAATTCAGGCCTGATTTCTTGTGGCTTT
TGTGATTTGATGGTGTCTTATTATTGATATTATCCACCATCAGGGTTTACTCTGCTGAGAGTCATACC

AAAGCTCTTTCAACCTGTAGCTCTCACATAATAGTTGTGGTCCTATTCTTTGTGCCTGCCCTCTTCATT
ACATCAGACCAGCCATAACTTTCCAGAAGATAAAGTGTGTTCTCTCTGTGCCATCATTGCTCCCAT
GTTTCAGTCTTCTATCTACATGCTGAGAAAGGTGGAGATGAAGAACGCTGTAAGGAAATGTGGTGTCTAT
CAATTGCTTCTGGCAAGGAAGTAACTTGTATGAAAG

The GPCR9 protein encoded by SEQ ID NO:32 has 309 amino acid residues, and is presented using the one-letter code in Table 9B (SEQ ID NO:33). The SignalP, Psort and/or Hydropathy profile for GPCR9 predict that GPCR9 has a signal peptide and is likely to be localized at the endoplasmic reticulum membrane with a certainty of 0.6850 or to the plasma membrane with a certainty of 0.6400. The SignalP predicts a cleavage site at the sequence IWM-EN between amino acids 38 and 39 as indicated by the slash in Table 9B.

Table 9B. Encoded GPCR9 protein sequence (SEQ ID NO:33)

MENRNIVTVFILLGLSQNKNIIEVFWFVFCYIAIWM/ENFIIMISIMYIWLIDQPMYFFLNLYALSDLC
YISTVAPKLMIDLLTERKIVSYNNCMQLFITHFLGDIBIFILKAMAYDHYIAICKHLHYTIITKQSCN
TIIACCTGGFIHSASQFLLTIFLPFCGLNEIDQYFCYVYPLLKLARIDIYRIGFLVIVNSGLISLLAFV
ILMVSYYLILSTIRVYSAESHTKALSTCSSHIIVVVLFVFPALFIYIRPAITFPEDKVFVLFCAIAPMF
SLLIYMLRKVEMKNAVRKMWCHQLLLARK

The full amino acid sequence of the protein of the invention was found to have 140 of 300 amino acid residues (46%) identical to, and 193 of 300 residues (64%) positive with, the 302 amino acid residue odorant receptor A16 protein from *Mus musculus* (ptnr:TREMBLNEW-ACC:BAA86127) (E value = $1.0e^{-72}$).

Patp results include those listed in Table 9C.

Table 9C. Patp alignments of GPCR9

Sequences producing High-scoring Segment Pairs:			Smallest Sum Prob. P (N)
	Reading Frame	High Score	
patp:Y83390 Olfactory receptor protein OLF-5 - H. sapiens...	+1	801	5.9e-79
patp:Y83394 Olfactory receptor protein OLF-9 - H. sapiens...	+1	791	6.8e-78
patp:Y90877 Human G protein-coupled receptor GTAR11-3 ...	+1	766	3.0e-75
patp:Y90877 Human G protein-coupled receptor GTAR11-3 ...	+1	766	3.0e-75
patp:Y90875 Human G protein-coupled receptor GTAR11-1 ...	+1	753	7.2e-74
patp:Y90875 Human G protein-coupled receptor GTAR11-1 ...	+1	753	7.2e-74
patp:Y83387 Olfactory receptor protein OLF-2 - H. sapiens...	+1	741	1.3e-72

For example, a BLAST against Y83390, a 305 amino acid olfactory receptor protein from *Homo sapiens*, produced 158/305 (51%) identity, and 213/305 (69%) positives (E = $5.9e^{-79}$), with long segments of amino acid identity. WO 00/21985.

Further BLAST analysis produced the significant results listed in Table 9D. The disclosed GPCR9 protein (SEQ ID NO:33) has good identity with a number of olfactory receptor proteins.

Table 9D. BLAST results for GPCR9					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Gi 11496249 ref NP_067343.1 (AB030895)	Odorant receptor MOR18 <i>Mus musculus</i>	308	140/300 (46%)	185/300 (61%)	1e-59
Gi 11464995 ref NP_065261.1 AB030896)	Odorant receptor A16 <i>Mus musculus</i>	302	137/300 (45%)	185/300 (61%)	2e-59
Gi 423702 pir S29710	Olfactory receptor OR18 - rat	307	142/303 (46%)	186/303 (60%)	6e-57
Gi 11464993 ref NP_065260.1 (AB030894)	Odorant receptor MOR83 <i>Mus musculus</i>	308	133/297 (44%)	182/297 (60%)	7e-55
Gi 10644519 gb AAG21324.1 AF271051_1 (AF271051)	Odorant receptor <i>Mus musculus</i>	264	124/262 (47%)	169/262 (64%)	6e-51

This information is presented graphically in the multiple sequence alignment given in Table 9E (with GPCR9 being shown on line 1) as a ClustalW analysis comparing GPCR9 with related protein sequences.

Table 9E. Information for the ClustalW proteins:

- 1) GPCR9 (SEQ ID NO:33)
- 2) gi|11496249|ref|NP_067343.1| odorant receptor 16 *Mus musculus* (SEQ ID NO:78)
- 3) gi|11464995|ref|NP_065261.1| gene for odorant receptor A16 *Mus musculus* (SEQ ID NO:79)
- 4) gi|423702|pir|S29710 olfactory receptor OR18 - rat (SEQ ID NO:80)
- 5) gi|11464993|ref|NP_065260.1| gene for odorant receptor MOR83 *Mus musculus* (SEQ ID NO:81)
- 6) gi|10644519|gb|AAG21324.1|AF271051_1 odorant receptor *Mus musculus* (SEQ ID NO:70)

	10	20	30	40	50	60
GPCR9
Gi 11496249
Gi 11464995
Gi 423702
Gi 11464993
Gi 10644519
	70	80	90	100	110	120
GPCR9
Gi 11496249
Gi 11464995
Gi 423702
Gi 11464993
Gi 10644519

		130	140	150	160	170	180
GPCR9		DRYVAICKPLHYTTTMRHHCYVLVAVAVGGGTHSICHLIFQLPFCGPNVINHEFVCD					
gi 11496249		DRYVAICKPLHYTTTMRHHCYVLVAVAVGGGTHSICHLIFQLPFCGPNVINHEFVCD					
gi 11464995		DRYVAICKPLHYTTTMRHHCYVLVAVAVGGGTHSICHLIFQLPFCGPNVINHEFVCD					
gi 423702		DRYVAICKPLHYTTTMRHHCYVLVAVAVGGGTHSICHLIFQLPFCGPNVINHEFVCD					
gi 11464993		DRYVAICKPLHYTTTMRHHCYVLVAVAVGGGTHSICHLIFQLPFCGPNVINHEFVCD					
gi 10644519		DRYVAICKPLHYTTTMRHHCYVLVAVAVGGGTHSICHLIFQLPFCGPNVINHEFVCD					
		190	200	210	220	230	240
GPCR9		LYPLLELACTDNTYVIGELVVVNSGVICLNLFLMAASYIVILHSLRSHSAEGRRKALSTC					
gi 11496249		LYPLLELACTDNTYVIGELVVVNSGVICLNLFLMAASYIVILHSLRSHSAEGRRKALSTC					
gi 11464995		LYPLLELACTDNTYVIGELVVVNSGVICLNLFLMAASYIVILHSLRSHSAEGRRKALSTC					
gi 423702		LYPLLELACTDNTYVIGELVVVNSGVICLNLFLMAASYIVILHSLRSHSAEGRRKALSTC					
gi 11464993		LYPLLELACTDNTYVIGELVVVNSGVICLNLFLMAASYIVILHSLRSHSAEGRRKALSTC					
gi 10644519		LYPLLELACTDNTYVIGELVVVNSGVICLNLFLMAASYIVILHSLRSHSAEGRRKALSTC					
		250	260	270	280	290	300
GPCR9		SSHILVVVILFFVPCIFMYRPSSTILSIDKLVAVFYCHITPMLNPLIYTLRNEEVKDMARK					
gi 11496249		SSHILVVVILFFVPCIFMYRPSSTILSIDKLVAVFYCHITPMLNPLIYTLRNEEVKDMARK					
gi 11464995		SSHILVVVILFFVPCIFMYRPSSTILSIDKLVAVFYCHITPMLNPLIYTLRNEEVKDMARK					
gi 423702		SSHILVVVILFFVPCIFMYRPSSTILSIDKLVAVFYCHITPMLNPLIYTLRNEEVKDMARK					
gi 11464993		SSHILVVVILFFVPCIFMYRPSSTILSIDKLVAVFYCHITPMLNPLIYTLRNEEVKDMARK					
gi 10644519		SSHILVVVILFFVPCIFMYRPSSTILSIDKLVAVFYCHITPMLNPLIYTLRNEEVKDMARK					
		310					
GPCR9		MMCHQLLLARK					
gi 11496249		MMCHQLLLARK					
gi 11464995		MMCHQLLLARK					
gi 423702		MMCHQLLLARK					
gi 11464993		MMCHQLLLARK					
gi 10644519		MMCHQLLLARK					

The presence of identifiable domains in GPCR9 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR9 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 9F with the statistics and domain description. The results indicate that GPCR9 contains the 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family) domain (as defined by Interpro) at amino acid positions residues 56-234. This indicates that the sequence of GPCR9 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

Table 9F. DOMAIN results for GPCR9

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) Length = 377 (SEQ ID NO:45).

Score = 73.6 bits (179), Expect = 1e-14

GPCR9|.....|.....|.....|.....|.....|.....|.....|.....|.....
-----PMYFFFNYLALSDCYISTVAPEKLMIDLTERKIVSYNNQMIO
TM7 GNVLCMAVSREKALQTTTNLYLVSLAVADLLVALVMPWVVYLEVVGWEKFSRIHCDF

GPCR9 LFIITHFLGDIEIFILKEMAYDHYIALCKHLHYTIITIKOSCNTIIACCTGGFIHSASQ
TM7 VTIIDVMCTASILLNCAISIRRYTAVAMPMLNTRYSSRRRTVMIAIVVWLSTIISCPM

GPCR9 FLL-TIFLPFCGLNEIDQYFCYVVELLKLRADIDYKRGFL--VIINSGLSLDAFVILM
TM7 LFGLNNTDQNE---CIIANE---AFVYYSIVSFYV---PFIVTLLEYI

GPCR9 VSVYYILLSTIRVYSAESHTKA-----
TM7 KIIYIVLRRRRKRVTNRSSRAFRANLKAPLKGNCTHPEDMKLCTVIMKSNGSFPVNRRRV

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dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and treatment of Albright hereditary osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR9 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR9 Antibodies" section below.

For example the disclosed GPCR9 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR9 epitope is from about amino acids 75 to 100. In another embodiment, a GPCR9 epitope is from about amino acids 115 to 145. In additional embodiments, GPCR9 epitopes are from amino acids 225 to 240 and from amino acids 290 to 310. These novel proteins can also be used to develop assay system for functional analysis.

GPCR10

GPCR10 includes a family of three similar nucleic acids and three similar proteins disclosed below. The disclosed nucleic acids encode GPCR, OR-like proteins.

GPCR10a

5 The disclosed novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation’s sequence file for GPCR probe or homolog, run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of
10 similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. GPCR10a is a 948 bp long nucleic acid (also referred to as 6-L-19-A) as shown in Table 10A (SEQ ID NO:34). An ORF begins with an ATG initiation codon at nucleotides 7-9 and ends with a TAA codon at nucleotides 934-936. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 10A, and
15 the start and stop codons are in bold letters.

Table 10A. GPCR10a Nucleotide Sequence (SEQ ID NO:34)

TGAGAAATGGAAATCAAAACAATGTGACTGAATTCATTCTTCTGGGTCTCACAGAGAACCTGGAGCTGT
GGAAATATTTTCTGCTGTGTTTCTTGTCATGTATGTAGCCACAGTGCTGGAAATCTACTTATTGTGGT
AACTATTATCACAAGTCAGAGTCTGAGGTCACCTATGTATTTTTTCTTACCTTCTGTCCCTTTTGGAT
GTCATGTTCTCATCTGTCGTTGCCCCAAGGTATTGTAGACACCCTCTCCAAGAGCACTACCATCTCTC
TCAAAGGCTGCCTCAGGCTGTTTGTGGAGCATTTCTTGGTGGTGTGGGGATCATCTCTCTCACTGT
GATGGCCTATGACCGCTACGTGGCCATCTGTAAGCCCCGCACTACACGATCATCATGAGTCCACGGGTG
TGCTGCCTAATGGTAGGAGGGGCTTGGGTGGGGGATTATGCACGCAATGATACAACTTCTCTTCATGT
ATCAAATACCCTTCTGTGGTCCTAATATCATAGATCACTTTATATGTGATTGTTTCAGTTGTGACACT
TGCTGCACGGACACCCACATCCTGGGCCTCTTAGTTACCCTCAACAGTGGGATGATGTGTGTGGCCATC
TTTCTTATCTTAATTGCGTCTACACGGTCATCTATGCTCCCTGAAGTCTTACAGCTCTAAAGGGCGGC
ACAAAGCCCTCTCTACCTGCAGCTCCACCTCACGGTGGTGTATGTTCTTTGTCCCTGTATTTCTT
GTACATGAGGCCTGTGGTCACTCAGGATAGACAAGGCAATGGCTGTGTGAGTCAATCATCACACCC
ATGTTAAATCCCTTGATCTATACACTGAGGAATGCAGAGGTGAAAAGTGCCATGAAGAACTCTGGATGA
AATGGGAGGCTTTGGCTGGGAAATAACTGCAATGCTGA

20 The GPCR10a protein encoded by SEQ ID NO:34 has 309 amino acid residues, and is presented using the one-letter code in Table 10B (SEQ ID NO:35). The SignalP, Psort and/or Hydropathy profile for GPCR10a predict that GPCR10a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The SignalP predicts a cleavage site at the sequence VLE-NL, between amino acids 39 and 40, as indicated by the slash in Table 10B.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 625 of 908 bases (68%) identical to a 909 bp *Mus musculus* gene for odorant receptor A16 mRNA (GENBANK-ID: AB030896|acc:AB030896) (E= 3.0e-75).

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Table 10B. Encoded GPCR10a protein sequence (SEQ ID NO:35).

MENQNNVTEFILLGLTENLELWKIFSAVFLVMYVATVLE/NLLIVVTIITSQSLRSPMYFFLTFLSLLDVM
FSSVVAPKVIIVDTLSKSTTISLKGCLTQLFVEHFFGGVGIILLTMAYDRYVAICKPLHYTIIMSPRVCC
LMVGGAWVGGFMHAMIQLLFMYQIPFCGPNIIDHFICDLFQLLTACTDTHILGLLVTLNSGMMCVAIPL
ILIASYTVILCSLKSYSKGRHKALSTCSSHLTVVVLFFVPCIFLYMRPVVTHPIDKAMAVSDSIITPML
NPLIYTLRNAEVKSAMKKLWMKWEALAGK

The full amino acid sequence of the protein of the invention was found to have 183 of 302 amino acid residues (60%) identical to, and 232 of 302 residues (76%) positive with, the 307 amino acid residue OR18 odorant receptor protein from *Rattus sp.* (ptnr: TREMBLNEW-ACC:G264618).

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GPCR10b

GPCR10a (6-L-19-A) was subjected to an exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide GPCR10b, which is also referred to as 6-L-19-A1.

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The nucleotide sequence for GPCR10b (948 bp, SEQ ID NO:36) is presented in Table 10C. The nucleotide sequence differs from GPCR10a by one nucleotide change (numbered with respect to GPCR10a) T404 >C.

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Table 10C. GPCR10b Nucleotide Sequence (SEQ ID NO:36)

TGAGAAATGGAAATCAAACAATGTGACTGAATTCATTCTTCTGGGTCTCACAGAGAACCTGGAGCTGTGGAAATATT
TTCTGCTGTGTTTCTTGTCATGTATGTAGCCACAGTCTGGAAAATCTACTTATTGTGGTAACTATTATCACAAGTCAGA
GTCTGAGGTACCTATGTATTTTTTCTTACCTTCTGTCCCTTTGGATGTCATGTTCTCATCTGTGCGTTGCCCAAG
GTGATTGTAGACACCTCTCCAAGAGCACTACCATCTCTCAAAGGCTGCCTCACCCAGCTGTTGTGGAGCATTTCTT
TGGTGGTGTGGGGATCATCCTCCTCACTGTGATGGCCTATGACCGCTACGTGGCCATCTGTAAGCCCTGCACTACACGA

TCACCATGAGTCCACGGGTGTGCTGCCTAATGGTAGGAGGGGCTTGGGTGGGGGATTATGCACGCAATGATACAACTT
 CTCTTCATGTATCAATACCCCTTCTGTGGTCCTAATATCATAGATCACTTATATGTGATTGTTTCAGTTGTGACACT
 TGCTGCACGGACACCCACATCCTGGGCCTCTTAGTTACCCCAACAGTGGGATGATGTGTGTGGCCATCTTTCTTATCT
 TAATTGCGTCTACACGGTCATCCTATGCTCCCTGAAGTCTTACAGCTCTAAAGGGCGGCACAAAGCCCTCTCTACCTGC
 AGCTCCACCTACGGTGGTGTATTGTTCTTTGTCCCTGTATTTCTTGTACATGAGGCCTGTGGTCACTCACCCTCAT
 AGACAAGCAATGGCTGTGTGAGACTCAATCATCACCCCATGTTAAATCCCTTGATCTATACACTGAGGAATGCAGAGG
 TGAAGAGTGCCATGAAGAACTCTGGATGAAATGGGAGGCTTGGCTGGGAAATAACTGCAATGCTGA

The encoded GPCR10b protein is presented in Table 10D. The disclosed protein is 309 amino acids long and is denoted by SEQ ID NO:37. GPCR10b differs from GPCR10a by one amino acid residue: I133>T. Like GPCR10a, the Psort profile for GPCR10b predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a peptide is between amino acids 39 and 40, *i.e.*, between the amino acid sequence VLE-NL (shown as a slash in Table10D) based on the SignalP result.

Table 10D. Encoded GPCR10b protein sequence (SEQ ID NO:37)

MENQNNVTEFILLGLTENLELWKIFSAVFLVMYVATVLE/NLLIVVTIITSQSLRSPMYFFLTFLSLLD
 VMFSSVVPKVI VDTLSKSTTISLKGCLTQLFVEHFFGGVGIILLTVMAYDRYVAICKPLHYTITMSPR
 VCCLMVGGAWVGGFMHAMIQLLFMYQIPFCGPNIIDHFICDLFQLLLTACTDTHILGLLVTLNSGMMCV
 AIFLLIASYTVILCSLKSYSKGRHKALSTCSSHLTVVVLFFVPCIFLYMRPVVTHPIDKAMAVSDSI
 ITPMLNPLIYTLRNAEVKSAMKKLWMKWEALAGK

Table 10E presents the BLASTP results for GPCR10b.

Table 10E. BLASTP Results for GPCR10b

Score = 999 (351.7 bits), Expect = 1.2e-100, P = 1.2e-100 Identities = 182/302 (60%), Positives = 231/302 (76%) with PIR-ID:S29710 olfactory receptor OR18 - rat
 Score = 757 (266.5 bits), Expect = 5.2e-75, P = 5.2e-75 Identities = 144/298 (48%), Positives = 200/298 (67%) with ACC:O95013 WUGSC:H_DJ0855D21.1 PROTEIN - Homo sapiens (Human), 312 aa.
 Score = 757 (266.5 bits), Expect = 5.2e-75, P = 5.2e-75 Identities = 144/298 (48%), Positives = 200/298 (67%) with ACC:O95013 WUGSC:H_DJ0855D21.1 PROTEIN - Homo sapiens (Human), 312 aa.
 Score = 667 (234.8 bits), Expect = 1.1e-64, P = 1.1e-64 Identities = 131/300 (43%), Positives = 194/300 (64%), Frame = +1 with ACC:O43749 OLFACTORY RECEPTOR - Homo sapiens (Human), 312 aa.

GPCR10c

Another nucleotide sequence resulted when GPCR10a (6-L-19-A) was subjected to an exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that

is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries.

These primers were then employed in PCR amplification based on the following pool of human cDNAs: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain - hippocampus, brain - substantia nigra, brain - thalamus, brain -whole, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. Usually the resulting amplicons were gel purified, cloned and sequenced to high redundancy. The resulting sequences from all clones were assembled with themselves, with other fragments in CuraGen Corporation's database and with public ESTs. Fragments and ESTs were included as components for an assembly when the extent of their identity with another component of the assembly was at least 95% over 50 bp. In addition, sequence traces were evaluated manually and edited for corrections if appropriate. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide the sequence reported below, which is designated as Accession Number 6-L-19-A-da1, or GPCR10c.

The nucleotide sequence for GPCR10c (943 bp, SEQ ID NO:38) is presented in Table 10F. The GPCR10c nucleotide sequence differs from GPCR10a by having six fewer nucleotides at the 5' end and two nucleotide changes: (numbered with respect to GPCR10a) G466>A and C834>T.

Table 10F. GPCR10c Nucleotide Sequence (SEQ ID NO:38)

```
ATGGAAATCAAACAATGTGACTGAATTCATTCTTCTGGGTCTCACAGAGAACCTGGAGCTGTGGAAAA
TATTTCTGCTGTGTTTCTTGTGTCATGTATGTAGCCACAGTGCTGGAAATCTACTTATGTGGTAACTAT
TATCACAAGTCAGAGTCTGAGGTCACCTATGTATTTTTTCTTACCTTCTTGTCCCTTTTGGATGTCATG
TTCTCATCTGTCGTTGCCCAAGGTGATTGTAGACACCCTCTCCAAGAGCACTACCATCTCTCTCAAAG
GCTGCCTCACCCAGCTGTTTGTGGAGCATTTCTTGGTGGTGTGGGGATCATCCTCCTCACTGTGATGGC
CTATGACCGCTACGTGGCCATCTGTAAGCCCTGCACTACACGATCATCATGAGTCCACGGGTGTGCTGC
CTAATGGTAGGAGGGCTTGGGTGGGGGATTTATGCACACAATGATACAACCTTCTCTCATGTATCAAA
TACCCTTCTGTGGTCCTAATATCATAGATCACTTTATATGTGATTGTTTCAGTTGTGACACTTGCCTG
CACGGACACCCACATCCTGGGCCTCTTAGTTACCCTCAACAGTGGGATGATGTGTGTTGGCCATCTTCTT
ATCTTAATTGCGTCTACACGGTCATCTATGCTCCCTGAAGTCTTACAGCTCTAAAGGGCGGCACAAAG
CCCTCTTACTGCAGCTCCACCTCACGGTGGTGTATTGTTCTTGTCCCCTGTATTTCTTGTACAT
GAGGCTGTGGTCACTCACCCATAGACAAGGCAATGGCTGTGTGAGACTCAATCATACCCATGTTA
AATCCCTTGATCTATACACTGAGGAATGCAGAGGTGAAAGTGCCATGAAGAACTCTGGATGAAATGGG
AGGCTTTGGCTGGGAAATAACTGCAATGCTGA
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The coding region of GPCR10c is from nucleotide 1 to 928, giving the encoded GPCR10c protein, as presented in Table 10G. The disclosed protein is 309 amino acids long

and is denoted by SEQ ID NO:83. GPCR10c differs from GPCR10a by one amino acid residue: A154>T. Like GPCR10a, the Psort profile for GPCR10c predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a peptide is between amino acids 39 and 40, *i.e.*, at the slash in the amino acid sequence VLE-NL (shown as a slash in Table10G) based on the SignalP result.

Table 10G. Encoded GPCR10c protein sequence (SEQ ID NO:83)

MENQNNVTEFILLGLTENLELWKIFSAVFLVMYVATVLE/NLLIVVTIITSQSLRSPMYFFLTFLSLLDVM
FSSVVAPKVIIVDTLSKSTTISLKGCLTQLFVEHFFGGVGIIILLTVMAYDRYVAICKPLHYTIIMSPRVCC
LMVGGAWVGGFMHTMIQLLFMYQIPFCGPNIIDHFCDLFQLLTACTDTHILGLLVTINSGMMCVAIFL
ILIASYTVILCSLKSYSKGRHKALSTCSSHLTVVVLFFVPCIFLYMRPVVTHPIDKAMAVSDSIITPML
NPLIYTLRNAEVKSAMKKLWMKWEALAGK

Possible SNPs found for GPCR10 are listed in Table10H.

Table 10H: SNPs

Base Position	Base Before	Base After
65	T	A(2)
120	T	Gap(2)
147	T	C(2)
234	A	G(3)
412	T	C(7)
471	G	A(2)
814	A	G(3)

Patp results include those listed in Table 10I.

Table 10I. Patp alignments of GPCR10

Sequences producing High-scoring Segment Pairs:				Smallest
		Reading Frame	High Score	Sum Prob. P (N)
patp:Y90872	Human G protein-coupled receptor GTAR14-1 . . .	+1	796	2.0e-78
patp:Y90872	Human G protein-coupled receptor GTAR14-1 . . .	+1	796	2.0e-78
patp:Y92364	G protein-coupled receptor protein 4 H. sapiens. .	+1	788	1.4e-77
patp:Y90874	Human G protein-coupled receptor GTAR14-5 . . .	+1	703	1.4e-68
patp:Y90874	Human G protein-coupled receptor GTAR14-5 . . .	+1	703	1.4e-68
patp:R27868	Odorant receptor clone F5 - Rattus rattus, . . .	+1	683	1.9e-66
patp:Y90873	Human G protein-coupled receptor GTAR14-3 . . .	+1	671	3.5e-65

For example, a BLAST against Y90872, a 313 amino acid G-protein coupled receptor protein (GTAR14-1) from *Homo sapiens*, produced 148/297 (49%) identity, and 206/297 (69%) positives (E = 2.0e-78), with long segments of amino acid identity, as shown in Table 10J. WO 00/21999.

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Table 10J. Alignment of GPCR10 with Y90872 (SEQ ID NO:89)	
Length = 313 Plus Strand HSPs: Score = 796 (280.2 bits), Expect = 2.0e-78, P = 2.0e-78 Identities = 148/297 (49%), Positives = 206/297 (69%), Frame = +1	
GPCR10	3 NQNNVTEFILLGLTENLELWKIFSAVFLVMYVATVLENLLIVVTIITSQSLRSPMYFFLT 62
Y90872:	5 NQTRVTEFVFLGLTDNRVLEMLFFMAFSAIYMLTSGNIIIIATVFTPSLHTPMYFFLS 64
GPCR10:	63 FLSLLDVMFSSVVPKVIIVDTLSKSTTISLKGCLTQLFVEHFFGGVGIILLTVMAYDRYV 122
Y90872:	65 NLSFIDICHSSVTVPKMLEGLLLERKTISFDNCITQLFFLHLFACAEIFLLIIVAYDRYV 124
GPCR10:	123 AICKPLHYTIIMSPRVCCLMVGGAWVGGFMHAMIQLLFMYQIPFCGPNIIDHFICDLFQL 182
Y90872:	125 AICTPLHYPNVMNMRVCIQLVFALWLGTVHSLGQTFLTIRLPYCGPNIIDSYFCDVPLV 184
GPCR10:	183 LTLACTDTHILGLLVTLNSGMMCAIFLILIASYTVILCSLKSYSKGRHKALSTCSSHL 242
Y90872:	185 IKLACTDTYLTGILIVTNSGTISLSCFLAVVTSYMVILVSLRKHSABGRQKALSTCSAHF 244
GPCR10:	243 TVVVLFFVPCIFLYMRPVVTHPIDKAMAVSDSIITPMLNPLIYTLRNEVKSAMKKL 299
Y90872:	245 MVVALFFGPCIFIYTRPDTSFSDIKVSVFYTVTPLNPFIIYTLRNEEVKSAMKQL 301

10 The disclosed GPCR10 protein (SEQ ID NO:35) has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 10K. Unless specifically addressed as GPCR10a GPCR10b, or GPCR10c, any reference to GPCR10 is assumed to encompass all variants. Residue differences between any

15 GPCRX variant sequences herein are written to show the residue in the "a" variant and the residue position with respect to the "a" variant. GPCR residues in all following sequence alignments that differ between the individual GPCR variants are highlighted with a box and marked with the (o) symbol above the variant residue in all alignments herein. For example,

20 the protein shown in line 1 of Table 10L depicts the sequence for GPCR10a, and the positions where GPCR10b or GPCR10c differs are marked with a (o) symbol and are highlighted with a box. All GPCR10 proteins have significant homology to olfactory receptor (OR) proteins:

Table 10K. BLAST results for GPCR10

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Gi 11496249 ref NP_067343.1 (AB030895)	Odorant receptor MOR18 <i>Mus musculus</i>	308	184/306 (60%)	241/306 (78%)	6e-91
Gi 423702 pir S29710	OR OR18 - rat	307	183/302 (60%)	232/302 (76%)	2e-88
Gi 11464995 ref NP_065261.1 AB030896)	Odorant receptor A16 <i>Mus musculus</i>	302	175/302 (57%)	232/302 (75%)	8e-86
Gi 11464993 ref NP_065260.1 (AB030894)	Odorant receptor MOR83 <i>Mus musculus</i>	308	157/297 (52%)	208/297 (69%)	3e-72
Gi 10644517 gb AAG21323.1 AF271050_1 (AF271050)	Odorant receptor <i>Rattus norvegicus</i>	264	155/260 (59%)	202/260 (77%)	2e-71

This information is presented graphically in the multiple sequence alignment given in Table 10L (with GPCR10 being shown on line 1) as a ClustalW analysis comparing GPCR10 with related protein sequences.

Table 10L Information for the ClustalW proteins:

- 1) GPCR10 (SEQ ID NO:35)
- 2) gi|11496249|ref|NP_067343.1| odorant receptor 16 *Mus musculus* (SEQ ID NO:78)
- 3) gi|423702|pir||S29710 olfactory receptor OR18 - rat (SEQ ID NO:80)
- 4) gi|11464995|ref|NP_065261.1| gene for odorant receptor A16 *Mus musculus* (SEQ ID NO:79)
- 5) gi|11464993|ref|NP_065260.1| gene for odorant receptor MOR83 *Mus musculus* (SEQ ID NO:81)
- 6) gi|10644517|gb|AAG21323.1|AF271050_1 odorant receptor *Rattus norvegicus* (SEQ ID NO:82)

	10	20	30	40	50	60
GPCR10	MEINNN--VTEFIIILGLTENLELWKIFSAFLVIVYATVHENELIVVTITTSQSLRSPMY					
gi 11496249	MEIIPN--ITEFIMLGLSORPETQRLLFVVFIVYAVTVCGNMLIVVTITFSSSSIASPMY					
gi 423702	MGENNN--ITEFIIILGLTQDPDGRKALFVTEFLIYVITMGNLLIVVTIASPSLGSPMY					
gi 11464995	MDSPRN--VTEFIMLGLSONPQVQRMFLGFLIDVFLVSVCGNMLIITITFTSPILGSPMY					
gi 11464993	MGALNQTRVTEFIFLGLDHWLEILFEVPTIVTYMTLIGNFLIVVTIVFTPRLHNPMY					
gi 10644517	-----VCFVETLPMYLAIVLGNGLIVVTYNISKSLISPMY					
	70	80	90	100	110	120
GPCR10	FFLTFLLSLIDVMSVVAPKVIIVDTLSKSTTISLKGCTOLFVEHFFGCGVGIILLTVMAY					
gi 11496249	FFLSNLSFIDTCYSSSLAPKLIADSLVEGTFISMEGCVACLFGAHLGGVEIILLTVMAY					
gi 423702	FFLSLSLLDALSTAIKPKIADLLIQKTISFRACVSOLEIEHLEGGVILVILVAMAY					
gi 11464995	FFLSYLSFIDTCYSSCHTPKLIADSLHEGKRAISFEGCQACFFVAHLLGGTEIILLTVMAY					
gi 11464993	FFLSNLSFIDTCYSSVTVPKMLEGLLLEKRTISHDNCHLAQLFELHLFACSEIFLLTMAY					
gi 10644517	FFLSYLSLVEIILYSSIVVPKFITDLLEKIKTISLKGCLAQHFFHFFGVTLEIWLTVMAY					
	130	140	150	160	170	180
GPCR10	DRYVAICKPLHYTITMSPRVCCLMVGGAWGGFHHAMICILFMYQIPFCGPNVIDHFFCD					
gi 11496249	DRYVAICKPLHYTTTMRHICVVLVAVAWGGFHHSLVQILLIFQLPFCGPNVIDHFFCD					
gi 423702	DRYVAICKPLHYLAAMNRVCTLLIFAWTGGFHHSLICIVFYVYNLFPFCGPNVIDHFFCD					
gi 11464995	DRYVAICKPLHYTTTMRVCTVVLVAVAWGGIHHSTACFFLYLGLPFCGPNVIDHFFCD					
gi 11464993	DRYVAICTPLHYSNMMNRVCTLVFALWGGTTHSLVCTFTLTIRLPFCGPNVIDSFFCD					
gi 10644517	DRYVAICKPLHYTTMSRPCHRLVAGSWGGFHHSLICIFITIPLPFCGPNVIDHFFCD					
	190	200	210	220	230	240
GPCR10	LFQLLTLACTDTHLGLLVTLNSGMNCVAIFLLFASYTVIHLCSLKSYSKGRHKALSTC					

gi 11496249	LYPLLELACTNTYVIGLLVANSQVICHNFIMLAASYIVILHSLRSHSAEGRRKALSTC
gi 423702	MSPLLVLACTDTYFGLTVIANGSVNCLVIFILLGSGYCHILRSIRIQSQEGRRKALSTC
gi 11464995	LYPLLELACTDTYVIGLLVANSQVICHNFIMLAASYIVILHSLRSHSAEGRRKALSTC
gi 11464993	VPPVTKLACTDTYITCLLVNSNGTISIMCFIALVTSYIVILFSLRKKSAEGRRKALSTC
gi 10644517	LHPLFKLACTDTFVVGVIMFVNSGLXSMFPFLXLVSSYIVILYNLRNNSAEGRRKALSTC

	250	260	270	280	290	300
GPCR10	SSHLTVVVLFFVPCIFYYRFPVVTHPTDKAMAVSDSIITPMLNPLIYTLRNAEVKSAAMKK					
gi 11496249	GAHFTVVVIMFFVPCIFSYMRPSTILPIDKNMAVFYGIITPMLNPLIYTLRNEEVKDAEMKK					
gi 423702	SSHLTVVVLFFVPCIFYYRFPVYNFPIDKCTIVFYIITPMLNPLIYTLRNSSEIKSCMKK					
gi 11464995	GAHFTVVVIMFFVPCIFHYMRPSTLSIDKIIVAVFYCIITPMFNPLIYTLRNAEVKNAMKN					
gi 11464993	SAHFMVVVILFFGPCIFLYTRPDSSFSIDKVVSIFYIVVTPMLNPLIYTLRNEEVKTAMKH					
gi 10644517	ASHIMVVVLEFGSAIFLYTERPASTYTEDKLVAVFYIVVTPMNNPLIYTL-----					

	310
GPCR10	IMMKWEALAGK
gi 11496249	LFTRSEVVGA-
gi 423702	FWCKMLHAD--
gi 11464995	FWRK-----
gi 11464993	LRQRIRICS---
gi 10644517	-----

The presence of identifiable domains in GPCR10 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR10 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 10M with the statistics and domain description. The results indicate that this protein contains the following protein domains (as defined by Interpro) at the indicated positions: domain name 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family) at amino acid positions 39 to 213. This indicates that the sequence of GPCR10 has properties similar to those of other proteins known to contain this domain and similar to the properties of this domain.

Table 10M. DOMAIN results for GPCR10	
gnl Pfam pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) Length = 377 (SEQ ID NO:45).	
Score = 93.6 bits (231), Expect = 1e-20	
GPCR10	ENELIVVTITTSOSLRSPMYFFTFLLSLLDVMFSSVVAERVVDTHSKSTTISLKGCLTQ
TM7	GNVLYCMASREKALOTTNNYLVSLAVADLEIVALLVMEWVYLEVGEWKFSRIHCDIF
GPCR10	TFVHFHFGGVGHTLTVMAYDRYVAICKPEHYTIIM-SPRVCCIMVGGANVGGEMHMIQ
TM7	VTLDVMMCTASTINICALSIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVWLSITISCPM
GPCR10	LLF-MYQIPFCG-----PNIIDHFICDLFQL---LILACIDTHILGLLVTSNSGMMC
TM7	IFGLNNTDQNE-----CIIANP-----AFVVYSIVSFYV---PFIITLLIYI
GPCR10	VAIFLLI-----
TM7	KIYIVRRRRKRVNTRKSSRAFRANLKAPLKGNCTHPEDMKLCTVIMKSNGSFPVNRRRV

The similarity information for the GPCR10 protein and nucleic acid disclosed herein suggest that GPCR10 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon. The novel nucleic acid encoding GPCR10, and the GPCR10 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in used in the treatment of infections such as bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and treatment of Albright hereditary osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium,

dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and treatment of Albright hereditary osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. These materials are further useful in the generation of antibodies that bind immuno-specifically to the novel GPCR10 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR10 Antibodies" section below. For example the disclosed GPCR10 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR10 epitope is from about amino acids 5 to 15. In another embodiment, a GPCR10 epitope is from about amino acids 225 to 240. In additional embodiments, GPCR10 epitopes are from amino acids 260 to 270 and from amino acids 290 to 310. These novel proteins can also be used to develop assay system for functional analysis.

A summary of the GPCR10 nucleic acids and proteins of the invention is provided in Table 11.

TABLE 11: Summary Of Nucleic Acids And Proteins Of The Invention

Name	Tables	Clone; Description of Homolog	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO
GPCR1	1A, 1B, 1E, 1F, 1I, 1J	GPCR1a: ba113a10_B, olfactory receptor	1	2
		GPCR1b: ba32713_A, olfactory receptor	3	4
		GPCR1c: ba113a10_C, olfactory receptor	5	6
GPCR2	2A, 2B 2I, 2J	GPCR2a: 11612531_1, olfactory receptor	7	8
		GPCR2b: 11612531_1_da1, olfactory receptor	84	85
GPCR3	3A, 3B	GPCR3: ba145L22_B, olfactory receptor	9	10
GPCR4	4A, 4B, 4C, 4G, 4H	GPCR4a1: dj408b20_C, olfactory receptor	11	12
		GPCR4a2: dj408b20_C_da1, olfactory receptor	13	
		GPCR4a3: CG55358_03, olfactory receptor	16	17
GPCR5	5A, 5B, 5C, 5D 5G, 5H	GPCR5a1: 115-a-12-A, olfactory receptor	18	19
		GPCR5a2: 115-a-12-B, olfactory receptor	20	21
		GPCR5a3: 115-a-12-A_da1, olfactory receptor	22	23
GPCR6	6A, 6B	GPCR6: 6-L-19-C, olfactory receptor	24	25
GPCR7	7A, 7B	GPCR7: dj313i6_D olfactory receptor	28	29
GPCR8	8A, 8B	GPCR8: dj408b20_A, olfactory receptor	30	31
GPCR9	9A, 9B	GPCR9: 6-L-19-B, olfactory receptor	32	33
GPCR10	10A, 10B, 10C, 10D, 10F, 10G	GPCR10a: 6-L-19-A, olfactory receptor	34	35
		GPCR10b: 6-L-19-A1, olfactory receptor	36	37
		GPCR10c: 6-L-19-A_da1, olfactory receptor	38	83

GPCRX Nucleic Acids and Polypeptides

One aspect of the invention pertains to isolated nucleic acid molecules that encode GPCR_X polypeptides or biologically active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify GPCR_X-encoding nucleic acids (*e.g.*, GPCR_X mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of GPCR_X nucleic acid molecules. As used herein, the term “nucleic acid molecule” is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An GPCR_X nucleic acid can encode a mature GPCR_X polypeptide. As used herein, a “mature” form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring

polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full-length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an ORF described herein. The product “mature” form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a “mature” form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an ORF, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a “mature” form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term “probes”, as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term “isolated” nucleic acid molecule, as utilized herein, is one, which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an “isolated” nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated GPCR_X nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally

flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (e.g., brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein.

Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 as a hybridization probe, GPCR_X molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to GPCR_X nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue.

Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, or a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID

NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, or a portion of this nucleotide sequence (*e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an GPCR_X polypeptide). A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, thereby forming a stable duplex.

As used herein, the term “complementary” refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term “binding” means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to,

molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

10 A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of GPCR_X polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of
15 RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for an GPCR_X polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring
20 allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human GPCR_X protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, as well as a polypeptide
25 possessing GPCR_X biological activity. Various biological activities of the GPCR_X proteins are described below.

An GPCR_X polypeptide is encoded by the open reading frame ("ORF") of an GPCR_X nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted
30 by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered

as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human GPCR_X genes allows for the generation of probes and primers designed for use in identifying and/or cloning GPCR_X homologues in other cell types, *e.g.* from other tissues, as well as GPCR_X homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84.

Probes based on the human GPCR_X nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an GPCR_X protein, such as by measuring a level of an GPCR_X-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting GPCR_X mRNA levels or determining whether a genomic GPCR_X gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an GPCR_X polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of GPCR_X" can be prepared by isolating a portion SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 that encodes a polypeptide having an GPCR_X biological activity (the biological activities of the GPCR_X proteins are described below), expressing the encoded portion of GPCR_X protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of GPCR_X.

GPCR_X Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32,

34, 36, 38, and 84 due to degeneracy of the genetic code and thus encode the same GPCR_X proteins as that encoded by the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85.

In addition to the human GPCR_X nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the GPCR_X polypeptides may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the GPCR_X genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an GPCR_X protein, preferably a vertebrate GPCR_X protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the GPCR_X genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the GPCR_X polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the GPCR_X polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding GPCR_X proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the GPCR_X cDNAs of the invention can be isolated based on their homology to the human GPCR_X nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is

intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding GPCR proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5 °C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at T_m, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a

"naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* 78: 6789-6792.

Conservative Mutations

In addition to naturally-occurring allelic variants of GPCR_X sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 thereby leading to changes in the amino acid sequences of the encoded GPCR_X proteins, without altering the functional ability of said GPCR_X proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19,

21, 23, 25, 29, 31, 33, 35, 37, 83, and 85. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the GPCR_X proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the GPCR_X proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding GPCR_X proteins that contain changes in amino acid residues that are not essential for activity. Such GPCR_X proteins differ in amino acid sequence from SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85; more preferably at least about 70% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85; still more preferably at least about 80% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85; even more preferably at least about 90% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85; and most preferably at least about 95% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85.

An isolated nucleic acid molecule encoding an GPCR_X protein homologous to the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have

been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the GPCR_X protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an GPCR_X coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for GPCR_X biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved “strong” residues or fully conserved “weak” residues. The “strong” group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the “weak” group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant GPCR_X protein can be assayed for (i) the ability to form protein:protein interactions with other GPCR_X proteins, other cell-surface proteins, or biologically-active portions thereof, (ii) complex formation between a mutant GPCR_X protein and an GPCR_X ligand; or (iii) the ability of a mutant GPCR_X protein to bind to an intracellular target protein or biologically-active portion thereof; (*e.g.* avidin proteins).

In yet another embodiment, a mutant GPCR_X protein can be assayed for the ability to regulate a specific biological function (*e.g.*, regulation of insulin release).

Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the

nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (*e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire GPCR_X coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an GPCR_X protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85, or antisense nucleic acids complementary to an GPCR_X nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an GPCR_X protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the GPCR_X protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the GPCR_X protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of GPCR_X mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of GPCR_X mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of GPCR_X mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense

nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (*v*), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (*v*), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)*w*, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an GPCR_X protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (*e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other. See, e.g., Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, e.g., Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a chimeric RNA-DNA analogue (see, e.g., Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330).

Ribozymes and PNA Moieties

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave GPCR_X mRNA transcripts to thereby inhibit translation of GPCR_X mRNA. A ribozyme having specificity for an GPCR_X-encoding nucleic acid can be designed based upon the nucleotide sequence of an GPCR_X cDNA disclosed herein (*i.e.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an GPCR_X-encoding mRNA. See, e.g., U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* GPCR_X mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, GPCR_X gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the GPCR_X nucleic acid (e.g., the GPCR_X promoter and/or enhancers) to form triple helical structures that prevent transcription

of the GPCR_X gene in target cells. *See, e.g.,* Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the GPCR_X nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.,* the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. *See, e.g.,* Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (*e.g.,* DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained.

The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

PNAs of GPCR_X can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.,* inducing transcription or translation arrest or inhibiting replication. PNAs of GPCR_X can also be used, for example, in the analysis of single base pair mutations in a gene (*e.g.,* PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.,* S₁ nucleases (*see, Hyrup, et al., 1996. supra*); or as probes or primers for DNA sequence and hybridization (*see, Hyrup, et al., 1996, supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNAs of GPCR_X can be modified, *e.g.,* to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of GPCR_X can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.,* RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see, Hyrup, et al., 1996. supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.*, 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.,* 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine

phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g., Mag, et al., 1989. Nucl Acid Res 17: 5973-5988.* PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g., Finn, et al., 1996. supra.* Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g., Petersen, et al., 1975. Bioorg. Med. Chem. Lett. 5: 1119-11124.*

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g., for targeting host cell receptors in vivo*), or agents facilitating transport across the cell membrane (*see, e.g., Letsinger, et al., 1989. Proc. Natl. Acad. Sci. U.S.A. 86: 6553-6556; Lemaitre, et al., 1987. Proc. Natl. Acad. Sci. 84: 648-652; PCT Publication No. WO88/09810*) or the blood-brain barrier (*see, e.g., PCT Publication No. WO 89/10134*). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g., Krol, et al., 1988. BioTechniques 6:958-976*) or intercalating agents (*see, e.g., Zon, 1988. Pharm. Res. 5: 539-549*). To this end, the oligonucleotide may be conjugated to another molecule, *e.g., a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.*

GPCRX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of GPCR_X polypeptides whose sequences are provided in SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85 while still encoding a protein that maintains its GPCR_X activities and physiological functions, or a functional fragment thereof.

In general, an GPCR_X variant that preserves GPCR_X-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated GPCR_X proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also

provided are polypeptide fragments suitable for use as immunogens to raise anti-GPCR_X antibodies. In one embodiment, native GPCR_X proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, GPCR_X proteins are produced by recombinant DNA techniques.

- 5 Alternative to recombinant expression, an GPCR_X protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the GPCR_X protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language
10 "substantially free of cellular material" includes preparations of GPCR_X proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes preparations of GPCR_X proteins having less than about 30% (by dry
15 weight) of non-GPCR_X proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-GPCR_X proteins, still more preferably less than about 10% of non-GPCR_X proteins, and most preferably less than about 5% of non-GPCR_X proteins. When the GPCR_X protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium
20 represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the GPCR_X protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of GPCR_X proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the
25 language "substantially free of chemical precursors or other chemicals" includes preparations of GPCR_X proteins having less than about 30% (by dry weight) of chemical precursors or non-GPCR_X chemicals, more preferably less than about 20% chemical precursors or non-GPCR_X chemicals, still more preferably less than about 10% chemical precursors or non-GPCR_X chemicals, and most preferably less than about 5% chemical precursors or
30 non-GPCR_X chemicals.

Biologically-active portions of GPCR_X proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the GPCR_X proteins (*e.g.*, the amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85) that include fewer amino acids than the full-

length GPCR_X proteins, and exhibit at least one activity of an GPCR_X protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the GPCR_X protein. A biologically-active portion of an GPCR_X protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native GPCR_X protein.

In an embodiment, the GPCR_X protein has an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85. In other embodiments, the GPCR_X protein is substantially homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85, and retains the functional activity of the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the GPCR_X protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85, and retains the functional activity of the GPCR_X proteins of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85.

Determining Homology Between Two or More Sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See*, Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP

extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84.

5 The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of
10 nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least
15 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

Chimeric and Fusion Proteins

20 The invention also provides GPCR_X chimeric or fusion proteins. As used herein, an GPCR_X "chimeric protein" or "fusion protein" comprises an GPCR_X polypeptide operatively-linked to a non-GPCR_X polypeptide. An "GPCR_X polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an GPCR_X protein (SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85), whereas a
25 "non-GPCR_X polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the GPCR_X protein, *e.g.*, a protein that is different from the GPCR_X protein and that is derived from the same or a different organism. Within an GPCR_X fusion protein the GPCR_X polypeptide can correspond to all or a portion of an GPCR_X protein. In one embodiment, an GPCR_X fusion
30 protein comprises at least one biologically-active portion of an GPCR_X protein. In another embodiment, an GPCR_X fusion protein comprises at least two biologically-active portions of an GPCR_X protein. In yet another embodiment, an GPCR_X fusion protein comprises at least three biologically-active portions of an GPCR_X protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the GPCR_X polypeptide and the non-GPCR_X

polypeptide are fused in-frame with one another. The non-GPCRX polypeptide can be fused to the N-terminus or C-terminus of the GPCRX polypeptide.

In one embodiment, the fusion protein is a GST-GPCRX fusion protein in which the GPCRX sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant GPCRX polypeptides.

In another embodiment, the fusion protein is an GPCRX protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of GPCRX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an GPCRX-immunoglobulin fusion protein in which the GPCRX sequences are fused to sequences derived from a member of the immunoglobulin protein family. The GPCRX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an GPCRX ligand and an GPCRX protein on the surface of a cell, to thereby suppress GPCRX-mediated signal transduction *in vivo*. The GPCRX-immunoglobulin fusion proteins can be used to affect the bioavailability of an GPCRX cognate ligand. Inhibition of the GPCRX ligand/GPCRX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the GPCRX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-GPCRX antibodies in a subject, to purify GPCRX ligands, and in screening assays to identify molecules that inhibit the interaction of GPCRX with an GPCRX ligand.

An GPCRX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.*, Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN

MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An GPCR_X-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the GPCR_X protein.

5

GPCR_X Agonists and Antagonists

The invention also pertains to variants of the GPCR_X proteins that function as either GPCR_X agonists (*i.e.*, mimetics) or as GPCR_X antagonists. Variants of the GPCR_X protein can be generated by mutagenesis (*e.g.*, discrete point mutation or truncation of the GPCR_X protein). An agonist of the GPCR_X protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the GPCR_X protein. An antagonist of the GPCR_X protein can inhibit one or more of the activities of the naturally occurring form of the GPCR_X protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the GPCR_X protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the GPCR_X proteins.

Variants of the GPCR_X proteins that function as either GPCR_X agonists (*i.e.*, mimetics) or as GPCR_X antagonists can be identified by screening combinatorial libraries of mutants (*e.g.*, truncation mutants) of the GPCR_X proteins for GPCR_X protein agonist or antagonist activity. In one embodiment, a variegated library of GPCR_X variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of GPCR_X variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential GPCR_X sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of GPCR_X sequences therein. There are a variety of methods which can be used to produce libraries of potential GPCR_X variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential GPCR_X sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. *See, e.g.*, Narang, 1983. *Tetrahedron* 39: 3;

Itakura, *et al.*, 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, *et al.*, 1984. *Science* 198: 1056;
Ike, *et al.*, 1983. *Nucl. Acids Res.* 11: 477.

Polypeptide Libraries

5 In addition, libraries of fragments of the GPCR_X protein coding sequences can be
used to generate a variegated population of GPCR_X fragments for screening and subsequent
selection of variants of an GPCR_X protein. In one embodiment, a library of coding sequence
fragments can be generated by treating a double stranded PCR fragment of an GPCR_X
coding sequence with a nuclease under conditions wherein nicking occurs only about once
10 per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-
stranded DNA that can include sense/antisense pairs from different nicked products,
removing single stranded portions from reformed duplexes by treatment with S₁ nuclease,
and ligating the resulting fragment library into an expression vector. By this method,
expression libraries can be derived which encodes N-terminal and internal fragments of
15 various sizes of the GPCR_X proteins.

Various techniques are known in the art for screening gene products of combinatorial
libraries made by point mutations or truncation, and for screening cDNA libraries for gene
products having a selected property. Such techniques are adaptable for rapid screening of the
gene libraries generated by the combinatorial mutagenesis of GPCR_X proteins. The most
20 widely used techniques, which are amenable to high throughput analysis, for screening large
gene libraries typically include cloning the gene library into replicable expression vectors,
transforming appropriate cells with the resulting library of vectors, and expressing the
combinatorial genes under conditions in which detection of a desired activity facilitates
isolation of the vector encoding the gene whose product was detected. Recursive ensemble
25 mutagenesis (REM), a new technique that enhances the frequency of functional mutants in
the libraries, can be used in combination with the screening assays to identify GPCR_X
variants. *See, e.g.,* Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815;
Delgrave, *et al.*, 1993. *Protein Engineering* 6:327-331.

Anti-GPCR_X Antibodies

30 The invention encompasses antibodies and antibody fragments, such as F_{ab} or (F_{ab})₂,
that bind immunospecifically to any of the GPCR_X polypeptides of said invention.

An isolated GPCR_X protein, or a portion or fragment thereof, can be used as an
immunogen to generate antibodies that bind to GPCR_X polypeptides using standard

techniques for polyclonal and monoclonal antibody preparation. The full-length GPCR_X proteins can be used or, alternatively, the invention provides antigenic peptide fragments of GPCR_X proteins for use as immunogens. The antigenic GPCR_X peptides comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85 and encompasses an epitope of GPCR_X such that an antibody raised against the peptide forms a specific immune complex with GPCR_X. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of GPCR_X that is located on the surface of the protein (e.g., a hydrophilic region). As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation (*see, e.g.*, Hopp and Woods, 1981. *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle, 1982. *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety).

As disclosed herein, GPCR_X protein sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically-binds (immunoreacts with) an antigen, such as GPCR_X. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and F_{(ab')₂} fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human GPCR_X proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an GPCR_X protein sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 17, 19, 21, 23, 25, 29, 31, 33, 35, 37, 83, and 85, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly-expressed GPCR_X protein or a

chemically-synthesized GPCR_X polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against GPCR_X can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of GPCR_X. A monoclonal antibody composition thus typically displays a single binding affinity for a particular GPCR_X protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular GPCR_X protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see, e.g., Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see, e.g., Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see, e.g., Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the invention and may be produced by using human hybridomas (see, e.g., Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see, e.g., Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an GPCR_X protein (see, e.g., U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see, e.g., Huse, *et al.*, 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for an GPCR_X protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See, e.g., U.S. Patent No. 5,225,539. Antibody fragments

that contain the idiotypes to an GPCR_X protein may be produced by techniques known in the art including, but not limited to: (i) an F_(ab)₂ fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an F_(ab)₂ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent; and (iv) F_v fragments.

Additionally, recombinant anti-GPCR_X antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314 :446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559; Morrison(1985) *Science* 229:1202-1207; Oi, *et al.* (1986) *BioTechniques* 4:214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an GPCR_X protein is facilitated by generation of hybridomas that bind to the fragment of an GPCR_X protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an GPCR_X protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-GPCR_X antibodies may be used in methods known within the art relating to the localization and/or quantitation of an GPCR_X protein (*e.g.*, for use in measuring levels of the GPCR_X protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for GPCR_X

proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-GPCR_X antibody (*e.g.*, monoclonal antibody) can be used to isolate an GPCR_X polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-GPCR_X antibody can facilitate the purification of natural GPCR_X polypeptide from cells and of recombinantly-produced GPCR_X polypeptide expressed in host cells. Moreover, an anti-GPCR_X antibody can be used to detect GPCR_X protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the GPCR_X protein. Anti-GPCR_X antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

GPCR_X Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an GPCR_X protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a

bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, GPCR proteins, mutant forms of GPCR proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of GPCR proteins in prokaryotic or eukaryotic cells. For example, GPCR proteins can be

expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be

5 transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein

10 encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the

15 recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse

20 glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990)

25 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, e.g., Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the

30 nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (see, e.g., Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the GPCR_X expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, GPCR_X can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249:

374-379) and the α -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to GPCR_X mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.,* Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, GPCR_X protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.,* DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in

Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding GPCR_X or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) GPCR_X protein. Accordingly, the invention further provides methods for producing GPCR_X protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding GPCR_X protein has been introduced) in a suitable medium such that GPCR_X protein is produced. In another embodiment, the method further comprises isolating GPCR_X protein from the medium or the host cell.

Transgenic GPCR_X Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which GPCR_X protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous GPCR_X sequences have been introduced into their genome or homologous recombinant animals in which endogenous GPCR_X sequences have been altered. Such animals are useful for studying the function and/or activity of GPCR_X protein and for identifying and/or evaluating modulators of GPCR_X protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome

of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous GPCR_X gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing GPCR_X-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human GPCR_X cDNA sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human GPCR_X gene, such as a mouse GPCR_X gene, can be isolated based on hybridization to the human GPCR_X cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the GPCR_X transgene to direct expression of GPCR_X protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the GPCR_X transgene in its genome and/or expression of GPCR_X mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding GPCR_X protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an GPCR_X gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the GPCR_X gene. The GPCR_X gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84), but more preferably, is a non-human homologue of a human GPCR_X gene. For example, a mouse homologue of human GPCR_X gene of SEQ ID NOS:1,

3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 can be used to construct a homologous recombination vector suitable for altering an endogenous GPCR_X gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous GPCR_X gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous GPCR_X gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous GPCR_X protein). In the homologous recombination vector, the altered portion of the GPCR_X gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the GPCR_X gene to allow for homologous recombination to occur between the exogenous GPCR_X gene carried by the vector and an endogenous GPCR_X gene in an embryonic stem cell. The additional flanking GPCR_X nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.*, Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is then introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced GPCR_X gene has homologously-recombined with the endogenous GPCR_X gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89:

6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*. See, O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.*, a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g.*, the somatic cell) is isolated.

Pharmaceutical Compositions

The GPCR_X nucleic acid molecules, GPCR_X proteins, and anti-GPCR_X antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, 5 intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, 10 and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous 15 solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under 20 the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, 25 by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the 30 composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., an GPCR_X protein or anti-GPCR_X antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active
5 compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

10 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and
15 swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch;
20 a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g.,
25 a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid
30 derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express GPCR_X protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect GPCR_X mRNA (*e.g.*, in a biological sample) or a genetic lesion in an GPCR_X gene, and to modulate GPCR_X activity, as described further, below. In addition, the GPCR_X proteins can be used to screen drugs or compounds that modulate the GPCR_X protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of GPCR_X protein or production of GPCR_X protein forms that have decreased or aberrant activity compared to GPCR_X wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease (possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-GPCR_X antibodies of the invention can be used to detect and isolate GPCR_X proteins and modulate GPCR_X activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to GPCR_X proteins or have a stimulatory or inhibitory effect on, *e.g.*, GPCR_X protein expression or GPCR_X protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an GPCR_X protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity

chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. See, e.g., Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, e.g., nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (e.g., Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of GPCR_X protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an GPCR_X protein determined. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the GPCR_X protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the GPCR_X protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ¹²⁵I, ³⁵S, ¹⁴C, or ³H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by

determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of GPCR_X protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds GPCR_X to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR_X protein, wherein determining the ability of the test compound to interact with an GPCR_X protein comprises determining the ability of the test compound to preferentially bind to GPCR_X protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of GPCR_X protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the GPCR_X protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCR_X or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the GPCR_X protein to bind to or interact with an GPCR_X target molecule. As used herein, a "target molecule" is a molecule with which an GPCR_X protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an GPCR_X interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An GPCR_X target molecule can be a non-GPCR_X molecule or an GPCR_X protein or polypeptide of the invention. In one embodiment, an GPCR_X target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound GPCR_X molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with GPCR_X.

Determining the ability of the GPCR_X protein to bind to or interact with an GPCR_X target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the GPCR_X protein to bind to or interact with an GPCR_X target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca²⁺, diacylglycerol, IP₃, etc.), detecting catalytic/enzymatic activity of the target an appropriate

substrate, detecting the induction of a reporter gene (comprising an GPCR_X-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

5 In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an GPCR_X protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the GPCR_X protein or biologically-active portion thereof. Binding of the test compound to the GPCR_X protein can be determined either directly or indirectly as described above. In one such embodiment, the
10 assay comprises contacting the GPCR_X protein or biologically-active portion thereof with a known compound which binds GPCR_X to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR_X protein, wherein determining the ability of the test compound to interact with an GPCR_X protein comprises determining the ability of the test compound to
15 preferentially bind to GPCR_X or biologically-active portion thereof as compared to the known compound.

 In still another embodiment, an assay is a cell-free assay comprising contacting GPCR_X protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the
20 GPCR_X protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCR_X can be accomplished, for example, by determining the ability of the GPCR_X protein to bind to an GPCR_X target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of
25 GPCR_X protein can be accomplished by determining the ability of the GPCR_X protein further modulate an GPCR_X target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

 In yet another embodiment, the cell-free assay comprises contacting the GPCR_X protein or biologically-active portion thereof with a known compound which binds GPCR_X
30 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR_X protein, wherein determining the ability of the test compound to interact with an GPCR_X protein comprises determining the ability of the GPCR_X protein to preferentially bind to or modulate the activity of an GPCR_X target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of GPCR_X protein. In the case of cell-free assays comprising the membrane-bound form of GPCR_X protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of GPCR_X protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either GPCR_X protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to GPCR_X protein, or interaction of GPCR_X protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-GPCR_X fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or GPCR_X protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of GPCR_X protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the GPCR_X protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated GPCR_X protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well

plates (Pierce Chemical). Alternatively, antibodies reactive with GPCR_X protein or target molecules, but which do not interfere with binding of the GPCR_X protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or GPCR_X protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the GPCR_X protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the GPCR_X protein or target molecule.

In another embodiment, modulators of GPCR_X protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of GPCR_X mRNA or protein in the cell is determined. The level of expression of GPCR_X mRNA or protein in the presence of the candidate compound is compared to the level of expression of GPCR_X mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of GPCR_X mRNA or protein expression based upon this comparison. For example, when expression of GPCR_X mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of GPCR_X mRNA or protein expression. Alternatively, when expression of GPCR_X mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of GPCR_X mRNA or protein expression. The level of GPCR_X mRNA or protein expression in the cells can be determined by methods described herein for detecting GPCR_X mRNA or protein.

In yet another aspect of the invention, the GPCR_X proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with GPCR_X ("GPCR_X-binding proteins" or "GPCR_X-bp") and modulate GPCR_X activity. Such GPCR_X-binding proteins are also likely to be involved in the propagation of signals by the GPCR_X proteins as, for example, upstream or downstream elements of the GPCR_X pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for GPCR_X is fused to a

gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an GPCR_X-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with GPCR_X.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the GPCR_X sequences, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, or fragments or derivatives thereof, can be used to map the location of the GPCR_X genes, respectively, on a chromosome. The mapping of the GPCR_X sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, GPCR_X genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the GPCR_X sequences. Computer analysis of the GPCR_X sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be

used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the GPCRX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. See, e.g., D'Eustachio, *et al.*, 1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the GPCRX sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, see, Verma, *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for

marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

5 Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.*, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*,
10 1987. *Nature*, 325: 783-787.

 Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the GPCR_X gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals,
15 then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a
20 mutation and to distinguish mutations from polymorphisms.

Tissue Typing

 The GPCR_X sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with
25 one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

 Furthermore, the sequences of the invention can be used to provide an alternative
30 technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the GPCR_X sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The GPCR_X sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining GPCR_X protein and/or nucleic acid expression as well as GPCR_X activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant GPCR_X expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining

whether an individual is at risk of developing a disorder associated with GPCR_X protein, nucleic acid expression or activity. For example, mutations in an GPCR_X gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with GPCR_X protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining GPCR_X protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCR_X in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of GPCR_X in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting GPCR_X protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes GPCR_X protein such that the presence of GPCR_X is detected in the biological sample. An agent for detecting GPCR_X mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to GPCR_X mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length GPCR_X nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, and 84, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to GPCR_X mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting GPCR_X protein is an antibody capable of binding to GPCR_X protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a

detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect GPCR_X mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of GPCR_X mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of GPCR_X protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of GPCR_X genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of GPCR_X protein include introducing into a subject a labeled anti-GPCR_X antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting GPCR_X protein, mRNA, or genomic DNA, such that the presence of GPCR_X protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of GPCR_X protein, mRNA or genomic DNA in the control sample with the presence of GPCR_X protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of GPCR_X in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting GPCR_X protein or mRNA in a biological sample; means for determining the amount of GPCR_X in the sample; and means for comparing the amount of GPCR_X in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect GPCR_X protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant GPCRX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with GPCRX protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant GPCRX expression or activity in which a test sample is obtained from a subject and GPCRX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of GPCRX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant GPCRX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant GPCRX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant GPCRX expression or activity in which a test sample is obtained and GPCRX protein or nucleic acid is detected (*e.g.*, wherein the presence of GPCRX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant GPCRX expression or activity).

The methods of the invention can also be used to detect genetic lesions in an GPCRX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an GPCRX-protein, or the misexpression of the GPCRX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (i) a

deletion of one or more nucleotides from an GPCR_X gene; (ii) an addition of one or more nucleotides to an GPCR_X gene; (iii) a substitution of one or more nucleotides of an GPCR_X gene, (iv) a chromosomal rearrangement of an GPCR_X gene; (v) an alteration in the level of a messenger RNA transcript of an GPCR_X gene, (vi) aberrant modification of an GPCR_X gene, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an GPCR_X gene, (viii) a non-wild-type level of an GPCR_X protein, (ix) allelic loss of an GPCR_X gene, and (x) inappropriate post-translational modification of an GPCR_X protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an GPCR_X gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the GPCR_X-gene (*see*, Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an GPCR_X gene under conditions such that hybridization and amplification of the GPCR_X gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see*, Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see*, Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Q β Replicase (*see*, Lizardi, *et al.*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an GPCR_X gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in GPCR_X can be identified by hybridizing a sample and control nucleic acids, *e.g.,* DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. *See, e.g.,* Cronin, *et al.*, 1996. *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in GPCR_X can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al., supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the GPCR_X gene and detect mutations by comparing the sequence of the sample GPCR_X with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.,* Naeve, *et al.*, 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (*see, e.g.,* PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the GPCR_X gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.,* Myers, *et al.*, 1985. *Science* 230: 1242. In general, the

art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type GPCR_X sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S₁ nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, e.g., Cotton, *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.*, 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in GPCR_X cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. See, e.g., Hsu, *et al.*, 1994. *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an GPCR_X sequence, e.g., a wild-type GPCR_X sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, e.g., U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in GPCR_X genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. See, e.g., Orita, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA*: 86: 2766; Cotton, 1993. *Mutat. Res.* 285: 125-144; Hayashi, 1992. *Genet. Anal. Tech. Appl.* 9: 73-79. Single-stranded DNA fragments of sample and control GPCR_X nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one

embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g., Keen, et al., 1991. Trends Genet. 7: 5.*

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g., Myers, et al., 1985. Nature 313: 495.* When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g., Rosenbaum and Reissner, 1987. Biophys. Chem. 265: 12753.*

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g., Saiki, et al., 1986. Nature 324: 163; Saiki, et al., 1989. Proc. Natl. Acad. Sci. USA 86: 6230.* Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g., Gibbs, et al., 1989. Nucl. Acids Res. 17: 2437-2448*) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g., Prossner, 1993. Tibtech. 11: 238*). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g., Gasparini, et al., 1992. Mol. Cell Probes 6: 1.* It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g., Barany, 1991. Proc. Natl. Acad. Sci. USA 88: 189.* In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an GPCR_X gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which GPCR_X is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on GPCR_X activity (*e.g.*, GPCR_X gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of GPCR_X protein, expression of GPCR_X nucleic acid, or mutation content of GPCR_X genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be

differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of GPCR_X protein, expression of GPCR_X nucleic acid, or mutation content of GPCR_X genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an GPCR_X modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCR_X (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase GPCR_X gene expression, protein levels, or upregulate GPCR_X activity, can be monitored in clinical trials of subjects exhibiting decreased GPCR_X gene expression, protein levels, or downregulated GPCR_X activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease GPCR_X gene expression, protein levels, or downregulate GPCR_X activity, can be monitored in clinical trials of subjects exhibiting increased GPCR_X gene expression, protein levels, or upregulated GPCR_X activity. In such clinical trials, the expression or activity of GPCR_X and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including GPCR_X, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates GPCR_X activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of GPCR_X and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of GPCR_X or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an GPCR_X protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the

level of expression or activity of the GPCR α protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the GPCR α protein, mRNA, or genomic DNA in the pre-administration sample with the GPCR α protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of GPCR α to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of GPCR α to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant GPCR α expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Osteodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below.

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to

"knockout" endogenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an

5 aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be

10 utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of

15 an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

20 **Prophylactic Methods**

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant GPCR_X expression or activity, by administering to the subject an agent that modulates GPCR_X expression or at least one GPCR_X activity.

25 Subjects at risk for a disease that is caused or contributed to by aberrant GPCR_X expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the GPCR_X aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type

30 of GPCR_X aberrancy, for example, an GPCR_X agonist or GPCR_X antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating GPCR_X expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of GPCR_X protein activity associated with the cell. An agent that modulates GPCR_X protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an GPCR_X protein, a peptide, an GPCR_X peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more GPCR_X protein activity. Examples of such stimulatory agents include active GPCR_X protein and a nucleic acid molecule encoding GPCR_X that has been introduced into the cell. In another embodiment, the agent inhibits one or more GPCR_X protein activity. Examples of such inhibitory agents include antisense GPCR_X nucleic acid molecules and anti-GPCR_X antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an GPCR_X protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) GPCR_X expression or activity. In another embodiment, the method involves administering an GPCR_X protein or nucleic acid molecule as therapy to compensate for reduced or aberrant GPCR_X expression or activity.

Stimulation of GPCR_X activity is desirable in situations in which GPCR_X is abnormally downregulated and/or in which increased GPCR_X activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (e.g., cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (e.g., preeclampsia).

Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Prophylactic and Therapeutic Uses of the Compositions of the Invention

The GPCR_X nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the GPCR_X protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the GPCR_X protein, and the GPCR_X protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

EQUIVALENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, which follow. In particular, it is

- 5 contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and
- 10 modifications considered to be within the scope of the following claims.